Serving Science and Society in the New Millennium: DOE's Biological and Environmental Research Program

U.S. Department of Energy
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Preface

Department of Energy (DOE) involvement in the life sciences has its roots in the research of the Atomic Energy Commission on the possible health effects of irradiation. The Atomic Energy Act of 1946 also mandated research on the beneficial applications, including medical, of nuclear technology. That research progressed from the determination of the health consequences of high levels of radiation exposure, on the basis of the study of Japanese atomic-bomb survivors, to more fundamental research on the mechanisms of biologic damage and repair after radiation exposure and other environmental insults associated with energy production. More recently, DOE has focused on molecular and cellular mechanisms of long-term health effects, including research on structural and computational biology, global climate change, the Human Genome Project, the human health consequences of various energy-related activities, and fields of biology related to energy production, such as plant biology. Many of those activities are administered by DOE's Office of Health and Environmental Research (OHER).

Future contributions of biologic sciences to the energy and environmental-quality missions of DOE could exceed those of the past. Biology could become as important a core discipline in DOE as physics has been. In fact, if the 20th century was the age of physics, many see the 21st century as the age of biology. In early 1997, DOE asked the National Research Council to cosponsor a symposium to assist the agency in identifying major gaps or research needs that are not being addressed by the current OHER program and in identifying opportunities to take advantage of the recent biologic advances in understanding the health effects of energy technologies and environmental remediation.

A symposium titled "Serving Science and Society into the New Millennium: The Legacy and the Promise" was held at the National Academy of Sciences on May 21-22, 1997. Speakers and panelists discussed the accomplishments and future of DOE's Biological and Environmental Research (BER) program. They also discussed a variety of multidisciplinary research activities, such as developing advanced medical diagnostic tools and treatments for human disease; assessing the health effects of radiation; tracking the regional and global movement of energy-related pollutants, and establishing the first human genome program. At the end of the symposium, 13 scientists who have been associated with the BER program and who have made significant contributions to its advancements and progress were honored. The proceedings volume includes the presentations made at the symposium.

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise in accordance with procedures for reviewing NRC and IOM reports approved by the NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will

assist the NRC in making the published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals, who are neither officials nor employees of the NRC, for their participation in the review of this report:

Dr. Rob Coppock, Washington, D.C.
Dr. William Ellett, Crofton, Maryland
Dr. James Tavares, Department of Energy
Dr. David Smith, Boonsboro, Maryland

It must be emphasized that responsibility for the final content of this volume rests entirely with the authors and the NRC.

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Serving Science and Society in the New Millennium: DOE's Biological and Environmental Research Program

Welcome

Kenneth I. Shine
President, Institute of Medicine
Washington, D.C.

Even though biology has made enormous progress in the last portion of the 20th century, the 20th century will be known as the century of physics and astronomy. Early in the century came $e = mc^2$ and quantum mechanics. Those concepts stimulated the application of physics to atomic energy. We believed that we could put someone on the moon, and we did it. The space program continues to excite the nation.

But the 21st century will be the century of the life sciences in all their ramifications. That includes not simply the use of molecular biology and genetics in human health, but the whole range of application of the life sciences—advances in agriculture, in animal husbandry, in cloning, in bioremediation to solve environmental problems, in the applications of biology in space, and so on. Dan Goldin, head of the National Aeronautics and Space Administration, has launched a new astrobiology program. Molecular biology might make it less expensive for the chemical industry to use organisms to generate new compounds than to produce them in large vats.

The life sciences include the behavioral sciences and the social sciences. When you consider that 50% of America's health-care bill is for illnesses that are produced by inappropriate behavior—alcohol and other substance abuse, violence, inappropriate sexual behavior—you can imagine that these are subjects in which there will also be enormous effort and that they include many related ethical issues.

The programs that we are celebrating today represent some of the very best in the transition of the life sciences to new frontiers. The next great frontier in the 21st century will not be in space, but will be the human mind—the capacity to relate to biology, emotions, behavior, thinking, and a variety of fundamental aspects of what makes intelligence. Some of the imaging programs that the Department of Energy (DOE) has supported use scanning technology to understand emotion, phantom pain, and various behavioral disorders.

We have seen a remarkable degree of reductionism in the last portion of the 20th century. We will need to begin to reintegrate what we know about genes and gene products into structure. This includes protein structure, the structure of the cell, and the structure of organs. DOE programs have included programs in structural biology that are on the cusp of this reintegration.

I hope you saw the synthesis pieces released by the Academy Complex, *Preparing for the 21st Century*, which contained 6 policy statements. One focused on the environment and the human future. The report says that, "for human societies to achieve a productive, healthful, and sustainable relationship with the natural world, the public and private sectors must make *environmental considerations an integral part of decision making*." We believe that, and we believe that studies undertaken by the Board on Sustainable Development, the Board on Biology, and the Commission on Life Sciences are relevant to that charge.

A major challenge for our society remains how to convey the idea of risk, not only for environmental but for medical reasons. The public understanding of risk remains fuzzy.

I am particularly pleased that the Academy Complex this summer will be hosting representatives of 11 academic health centers for a 5-day institute in which they will be joined by their colleagues from the public schools in a joint activity to apply the new science standards and to learn about hands-on teaching and how to achieve systemic change in public education. I hope you as individuals and collectively will support our efforts in K–12 education; without an educated public that understands risk, probability, and science, we will be hampered in carrying out major programs.

In the report on the environment (one of our synthesis pieces), there is an interesting discussion of the defect in the ozone layer and the work of Sherry Rowland. I mention that as a reminder that when Dr. Rowland, who received the Nobel Prize for this work, started to try to understand the events that led to the evidence of a hole in the ozone layer, he was not asking an applied question at all. He was interested in how some types of hydrocarbons are metabolized or degraded in the environment. Pursuing science—pursuing knowledge—eventually led him and his colleagues to the recognition of a problem in the atmosphere. It is in that context that the tradition of DOE—trying to support and sustain the highest-quality science, which can be translated into technologies that help all of us—makes the agency and your programs particularly important.

Congratulations on your work. I look forward to a very interesting meeting.

A Celebration of 50 Years of Health and Environmental Research

Martha A. Krebs
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U.S. Department of Energy
Washington, D.C.

It is a pleasure for me to be here to celebrate 50 years of health and environmental research. I have been the director of the Department of Energy (DOE) Office of Energy Research (OER) for a fairly long time. Any director becomes embedded in the history and traditions of his or her institution, and there is no question but that I am proud to be affiliated with OER. Today's events highlight the rich heritage of science that is the fruit of our investments over the last 50 years and of which I am proud to be a part.

We are the sponsors of the great contributions that the scientific community made during World War II and in the decades that followed. We have built on that success and advanced the frontiers. Through endeavors in high-energy physics and nuclear physics, materials science, and, of course, health and environmental research, we have established a standard of excellence and relevance for the important problems facing our nation now and in the future. As the director of OER, I believe that it is important to remind every audience that DOE is a science agency and that our science enables us to meet the energy challenges ahead. All too often, DOE is the forgotten science agency, despite its ranking among the top federal supporters of basic, applied, academic, and overall research. The department is the primary supporter of advanced research facilities, including our extensive scientific user facilities and world-class national laboratories. Among academic disciplines, DOE is the primary source of federal funding for physics and the other physical sciences and ranks among the top 5 for engineering, mathematics, and environmental and life sciences. The relevance of its diverse support to DOE missions has often come into question. It is the work and accomplishment of the people within OER to advance individual disciplines in the context of our overall energy and defense missions. But I want to remind this audience, as you get ready to listen to the particular contributions of the Office of Health and Environmental Research (OHER), that that support is just a part of a total portfolio that contributes broadly to science, as it advances energy security, environmental quality, and stockpile stewardship.

A few years ago, the contributions of DOE were less clear in Washington and even among academic communities. There were always the questions, Why the life sciences? Why is DOE doing this? What difference does it make? I think that what this audience knows, what you are celebrating here today and what will be detailed later, is the answer to those questions. It is important to recognize that if DOE were not making its investments, critical fields of scientific research would lack support. One example is the environmental research associated with energy use and ultimately climate change. This work includes climate modeling and some remarkable experimental programs, from atmospheric-radiation measurements to free-access CO_2 experiments. These experiments are changing how science is examining the world around us by combining traditional experimental approaches and introducing new instrumentation, data-gathering techniques, and computation capability.

The DOE scientific-user facilities are perhaps our most unusual contribution to science. Our synchrotrons, with some advances in nuclear magnetic resonance techniques, are helping to redefine structural biology. In addition, nuclear medicine has been recognized, from the very beginning, as a major contribution of DOE and its predecessor agencies. If DOE had disappeared, the loss of nuclear medicine would have been felt by every American family.

One of the most-exciting news stories of the last year was the validation of the existence of a third form of life: the Archaea. That validation was made possible by the investments and shared vision of DOE and the National Institutes of Health, driven by the insight of Charles DeLisi, to pursue genome sequencing. It is a testament to the capabilities and resources that DOE, with its laboratories and its principle investigators, uses to carry out such grand projects. Without that capability, without that vision, we would not have been in a position to fund The Institute for Genomic Research, to sequence one of the extremophiles and to learn that this creature was truly unlike any previously known.

The story of the microbial genome is intriguing. It also provides a justification of our approach to science by demonstrating how breakthroughs happen in an organization like OER. Our ability to support excellent science with casual connections to our energy mission has, in turn, advanced our mission in surprising ways. The human-genome project and the microbial extremophiles are 2 examples. These programs seem as remote from each other as they are from our energy mission. However, they come together in a discovery that creates a mind-bending experience in the scientific community: a new branch of life. In OER, we see the potential for equally important effects on energy and the environment that will continue to justify the kinds of investments that we have made in these programs.

Earlier here, Kenneth Shine commented that the next century will be the century of biology. As a physicist, I am less than happy about that idea, but I *am* intrigued. Yesterday, I spent the morning with a group of CEOs at the American Chemical Society. The purpose of the meeting was to stimulate ideas for the 75th anniversary of *Chemical & Engineering News*. In a focus group, we discussed the future of the chemical industry. Our final task was to predict some of the important trends or possible events in the chemical industry from a scientific or institutional perspective. As we went around the room, several people commented on the connections between material science, chemistry, and biology. The group explored discoveries that we might expect from these connections and their effect on world markets. We also discussed the effects of the Internet in connecting the researchers' communities and enabling the discoveries we identified. The use of large databases and collaborations across institutions and the ability to do science "at a distance" are expected to change not only the conduct of research, but the character and efficiency of innovation across the chemical industry.

I believe that those changes are taking place throughout science and are bridging the gaps between disciplines. In energy research, particularly in biologic and environmental research, we are leading this evolution in science. Fifty years ago, 25 years ago, and today we have been exploring the connections between the biologic sciences, the physical sciences, engineering, and computation in ways that bring together institutions, disciplines, and researchers. The technologic challenges of our mission have required this cross-programming and continue to drive our efforts.

OHER has one of the most-balanced programs within OER. Through investments in universities and national laboratories, OHER uses and unifies diverse disciplines to support and carry out its program. OHER provides a model for success. I want you to know that I recognize and value the success of OHER. It has a tradition of excellence and partnership that we must build broadly on within DOE and beyond. I am very happy to be here with all of you to celebrate that tradition.

The Biological and Environmental Research Program at 50

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On May 17, 1997, at the commencement address at Morgan State University, President Clinton said that if the last 50 years were the age of physics, the next 50 years will be the age of biology. (In fact, he upped the second number 15 minutes later to make it a century.)

Throughout the 2 days of this symposium, you will be hearing several speakers and panelists about the accomplishments and the future of the Department of Energy's (DOE) Biological and Environmental Research (BER) program. Also, you will see the many posters and exhibits in the Great Hall, where you can dig into the details of specific elements of the program. Tomorrow afternoon, we will be honoring 13 of our major scientists who have been associated with the BER program and have made important contributions to its advancement and progress.

HISTORICAL BACKGROUND

My colleagues Benjamin Barnhart and Murray Schulman have dug into the archives of our office in the DOE to distill some history. Kenneth Shine spoke earlier about the history of the BER program, starting during World War II when a medical advisory committee under Stafford Warren developed health and safety policy and associated research activities for the Manhattan Project. In 1946, the Atomic Energy Act created the Atomic Energy Commission (AEC), which came into being on January 1, 1947. Part of the act dealt with improving our knowledge of the potential damaging effects of ionizing radiation while extending our fundamental knowledge of the interactions between radiation and living matter and ensuring distribution of isotopes for medical and biologic applications. Research in health physics and radiation biology got under way at Oak Ridge, Hanford, Los Alamos, and the University of Chicago.

In September 1947, AEC and the National Academy of Sciences created the Advisory Committee for Biology and Medicine, which promptly recommended the creation of a Division of Biology and Medicine in AEC. In fact, the precise date is September 24, 1947, which is perhaps the birthday of the BER program.

Parallel to the many biologic applications were many applications in the environmental sciences, starting from the pioneering work in radioecology that began in the early 1950s and including studies aimed at determining the fate of fallout through atmospheric, terrestrial, and marine media.

In 1974, the Energy Reorganization Act created the Energy Research and Development Administration (ERDA) for "environmental, physical, and safety research related to the development of energy sources and utilization technologies"; this expanded the scope of the old Division of Biology and Medicine's research to

include all aspects of energy production and use. Thus was formed the Division of Biomedical and Environmental Research; when DOE was created 3 years later, it gave birth to the Office of Health and Environmental Research (OHER), which is what we have been for the last 20 years. OHER joined the Office of Energy Research in 1981. For most of the time since ERDA became DOE, Congress has known us as the Biological and Environmental Research program, and that is how our budget is titled.

THE FUTURE

At the doorstep of the 21st century, the BER program is poised to make important contributions to the exciting scientific advances that lie ahead. Our vision is to bring revolutionary solutions to energy-related biologic and environmental challenges. We believe that a diverse research portfolio like ours drives science at the interface where most of the advances will take place. One example is the interface between the biologic sciences and the information sciences that will essentially bring a revolution to biology in the next century. Another is the interface between global environmental change and greenhouse gas emissions. Martha Krebs has already referred to some of the programs. Our global-change program will continue to provide the rigorous science needed to put climate-change prediction—in fact, global environmental-change prediction—on solid ground.

International greenhouse-gas agreements loom ahead, and the need to become more rigorous in investigating the ecologic effects of global environmental change will prompt the BER program to expand its efforts in looking at the effects of global environmental change. That is one subject in which we have pioneered and expect to play a major role in the years ahead.

The BER program engages a diverse set of performers. Of BER's fiscal year 1996 operating funds of $313 million, $167.6 million (53.5%) went to the national laboratories, $82.7 million (26.4%) to colleges, $12.9 million (4.1%) to nonprofit institutions, and $49.8 million (15.9%) to the private sector. Such diversity challenges headquarters and the advisory committees that serve us to provide guidance for the scientific activities.

SUMMARY

We are proud of the shining examples that we have had of interagency and intra-agency cooperation, such as the cooperation with the National Institutes of Health in the Human Genome Program and the collaborations with many sister agencies, such as the National Aeronautics and Space Administration, the National Oceanic and Atmospheric Administration, the National Science Foundation, the Environmental Protection Agency, and others in the Global Change Research Program. Even within the DOE, we have an excellent working relationship with the Office of Environmental Management in launching and promoting the Environmental Management Science Program. There are many interactions with other program offices in the Office of Energy Research, such as those with the Office of Technology and Computational Research and the Office of Basic Energy Sciences.

We are committed to pushing and promoting that kind of cooperation because we believe strongly that the science of the future will be interdisciplinary and multidisciplinary and will require this multiagency and multi-organizational approach to developing and promoting successful activities.

I end by reminding you that a Biological and Environmental Research program like ours—a diverse research portfolio—drives science at the interface where most of scientific advances will occur.

Keynote Address: Impact of Biotechnology and Environmental Research on Science and Society in the 21st Century

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It is a pleasure to be here at this 50th anniversary and to talk about paradigm changes—paradigm changes in science and in the relationship between science and society—and the role that the Department of Energy (DOE) has played in catalyzing some of these changes.

Back in 1985, I first had interaction with DOE in the context of the human-genome project. The first official meeting for the human-genome project was one that Bob Sinsheimer had organized at Santa Cruz to consider the possibility of a genomic institute there. He invited about 10 investigators to explore the possibility, and I went with enormous skepticism but within a very short period became a real convert. Of course, the issue that came up at the very beginning was how one could go from an idea and a passion to a program, and this was not a trivial consideration at all. Fortunately, at about the same time, Charles DeLisi in DOE had similar ideas, for reasons that are fascinating. One of my first interactions with DOE was my testifying in Congress. There were rumors that DOE and I were going to be testifying against Jim Watson or against David Baltimore; in the beginning, those were the enthusiastic opponents of the whole idea of the genome initiative. I give DOE enormous credit: Without the insight, energy and fortitude of DOE, and in particular Charles DeLisi, the human-genome project today in the United States would be very different.

For me, what has been most interesting about the human-genome project is the first of the paradigm changes that I will talk about: the idea that biology is an information science. I moved to the University of Washington about 4½ years ago, in part to create a new department and in part to advocate paradigm changes both in science and in the scientist's responsibility to society.

I was fortunate to meet Bill Gates, who supported the department that we started. At the 4-hour dinner at the Columbia Towers, he made the statement that 2 disciplines were going to dominate the next century: information sciences and the biologic sciences. That statement fascinated me for 2 reasons. First was the idea that both were information sciences—biology about various kinds of biologic information, and the information sciences were about the digitized information in the real world. Second was the idea that the 2 disciplines were on a collision course—that our ability to execute biology in the future is going to be determined by our capacity to interact with computer science and applied mathematics. In the last five years, we have seen an enormous increase in our capacity to decipher biologic information, and I think in the 21st century we will see an increasing ability to manipulate biologic information. In fact, the manipulation of biologic information is going to be the very basis of preventive medicine as we move into the 21st century.

I would like to talk about the types of biologic information as I see them. The first type is the information of our genes and chromosomes. It is digital information, exactly like the digital information of the information sciences except that it has a 4-letter language rather than a 2-letter language.

Two things are particularly interesting about this digital language. The first is that variation in a digital 4-letter code is capable of writing the software that has the capacity to create something as complicated as a human organism, which has many letters—perhaps 3 billion in the human genome.

The second interesting aspect of the digital language of chromosomes is that it is not a single strand, but 2 intertwining helical strands, and these strands, or strings, exhibit a molecular complementarity with one another. The pairs of letters—As and Ts, and Gs and Cs—always match up in the 2 strands. This produces one of the fundamental and most fascinating traits of this type of information: we can break our very large chromosomes into small pieces and separate the strands from one another, and they can reassociate or find one another. That molecular complementarity is at the heart of how chromosomes work in transmitting information for life and at the heart of how many of the critical diagnostic techniques of the next century will lead to preventive medicine.

The chromosomes contain units of information called genes. Different genes can be expressed in different cells. The human-genome project has brought us an enormous capacity to assess the kind of information that is expressed in different cells, to convert this expressed information into DNA copies, and to analyze in a normal cell or in the tumor cell how the information varies. The second type of biologic information is the information that ensues when we take an intermediate string of messenger RNA and make a final string of it—a protein string of 20 letters that leads to a 3-dimensional structure. The 3-dimensional structure of a protein represents a molecular machine, and it is the 100,000 or so human genes that make (to a first approximation) 100,000 or so different molecular machines; and this is what gives the body shape and form that catalyzes the chemistry of life.

At the protein level, there are 2 fascinating questions: (1) given a linear protein string, can we predict the 3-dimensional structure that will be initiated? (2) given the 3-dimensional structure, can we figure out how it executes its function? Those are 2 enormous challenges.

The third type of information, which I think will be the information of the future, is the information that arises from complex systems and networks. Of the 10 to 12 neurons with their 10 to 15 connections, we can take a single nerve cell, study it for 20 years, and know virtually everything it does, but it won't tell us anything about the fascinating fundamental properties of this system—memory, consciousness, and the ability to learn—because they are properties of the network as a whole. How do we approach the study of systems? We can't do it by studying individual components. We need the tools, the capacity, the computational wherewithal to analyze systems in toto.

If we think about deciphering biologic information, there are 2 aspects for each of the 3 types of information. The sequence of DNA bases in our chromosomes is what the human-genome project is all about. But much deeper is the challenge of translating the information that 3.7 billion years of evolution has inscribed on our chromosomes. And so it is with proteins: It is one thing to determine structure, but it is quite another to understand how that structure executes its function. And so it is with systems as well: It is one thing to define elements and even to ascertain their linkages to one another, but it is quite another to understand how the system as a whole contributes properties (so-called emergent properties). This is the challenge and the opportunity that the human-genome project has given us—in spades.

I would like to talk about how biology and medicine are going to be transformed by the paradigm changes that we have seen. We have already noted that biology is an informational science and that there are 3 types of information. I would argue that the genome is a rosetta stone rather than a periodic table. "Periodic table" implies a single, unambiguous language, and that is not true of the chromosomes. Our chromosomes have a multiplicity of languages—some discreet and some overlapping—so the idea of a rosetta stone is more fitting.

To analyze the systems, we need to use high-throughput tools that have emerged from genomics, one of which is large-scale DNA sequencing. To give you an idea of how our ability to sequence DNA has changed when the first sequencing techniques were developed in the late 1970s, it took a year to sequence 10 nucleotides. We have machines today that in a year can sequence about 25 million nucleotides!

It is such tools that we are going to use to accomplish the 3 things necessary to define the elements, to define the linkage among them, and to define the emergent properties of complex systems. The challenge is to divide complicated systems into subsystems that we can analyze and whose properties reflect the properties of the entire system.

An idea that emerges naturally is that diseases, too, have to be approached through systems analysis, that they have to be approached as complicated systems to be studied. Two ideas come out of the systems analysis of

disease: that a given disease is not a single entity, but a series of entities; and that each of those entities—whether various forms of prostatic cancer or various forms of multiple sclerosis—is encoded by, predisposes one by, a multiplicity of genes, so we can write out a hypothetical stratification of, for example, prostatic cancer in which 3 subsets of predisposing genes cause 3 types of prostatic cancer. That is hypothetical, but it would lead to different clinical features—ultimately, to different kinds of therapeutic products and so forth. All that comes from the idea that diseases are systems.

Another idea that has emerged in part from the genome project is the unity of microorganisms. The idea of the unity of all life, that the informational pathways are shared by the simplest living organisms and all the way up to humans, belies their common evolutionary origin. If we define the simplest organisms and use them to define the information pathways, they will have profound implications for the information pathways present in humans. It is not just that they have given us insights into life. They have given us complete systems that we can use to analyze information pathways. A large fraction of the world of microbiology has been captivated by several of the microbial genomes that have been generated.

With the computational tools available, biology is going to be revolutionized. Our ability to acquire data, to store them, to analyze them, to model them, and to distribute them are all fundamental components of computational biology and applied mathematics that are essential to the biology of the future—if biology is information science.

The development of new tools has been important as new questions have come up in biology, and DOE has done a magnificent job in this regard. When more-sophisticated computational tools were needed for the human-genome project, DOE played a leading role in pioneering their development. In genomics, DOE is known for its commitment to fundamental science and the development of new tools, and this has turned out to be a very important part of biology.

A very-high-throughput tool that has been developed by a company called Affimetrix and Alan Blanchard in our laboratory has the capacity to put tens of thousands or even hundreds of thousands of DNA fragments on a small glass chip. Each fragment could represent, for example, one of the 100,000 or so human genes. By using molecular complementarity, one could analyze whether a particular gene in a particular cell is expressed. In a single test system, one could look at tens of thousands or hundreds of thousands of units of information. We have developed a technology for making 25 of these chips a day; each chip can have up to 150,000 DNA fragments of 20 letters, so if one knew them all, one could put representatives, information units, of all the different human genes on a single chip. That gives us the capacity not only to look at tumor cells and normal cells to see what the differences are, but also the capacity to carry out genetic mapping. We can use the fragments to map out genetic features and follow particular genetic markers or, for example, disease phenotypes. We can then localize genes, and there are techniques for identifying them and eventually understanding the information pathways that they are a part of and how they have functioned in causing particular diseases.

Biology is going to be changed when the complete human genome has been sequenced. In the year 2005, we expect to have identified the 100,000 or so human genes and to have transformed biology in a fundamental way: biologists of the past worked by starting with the function, finding the protein, and then identifying the corresponding gene, but in the future we will have all the genes and will have a desperate need to identify the function.

How can you go from a gene to a function? A new field, called functional genomics, presents one of the enormous challenges in biology today.

Part I

Enabling Research for Science and Technology: Biotechnology

Biotechnology and the Human Genome Project

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The Department of Energy (DOE) has played and is poised to continue to play a unique role in biology. This role is well symbolized by the Office of Health and Environmental Research (OHER). OHER is the only entity I know that covers the dimensions from the whole world, such as global climate change, through all kinds of environmental issues down through properties of individual DNA molecules. OHER constitutes a unique vertical integration, and I strongly encourage that this be continued.

My introduction to the Human Genome Project began at a meeting in Alta, Utah, in December 1984 that was cochaired by Mort Mendelson and Ray White. It was a very exciting meeting. It was called to deal with the issue of mutation detection in the Hiroshima-affected cohort, but it rapidly became an intense brainstorming session in which those present reached the consensus that if one wanted to detect mutations de novo in humans at the DNA level, one would have to have the analytic power to sequence the human genome.

Shortly after Alta, a few additional meetings were held, and a famous memorandum was written by Charles DeLisi to Al Trivelpiece, suggesting the possibility of a DOE-funded human-genome project. This year is the 10th anniversary of the Coldspring Harbor genome meetings, and it is appropriate that the cover of the booklet for that meeting showed a photocopy of the memo from DeLisi to Trivelpiece.

The most-remarkable thing about this project, looking back 10 or 15 years, is that, although all of us had a clear picture of the cost that would be involved and the number of base pairs that would be involved, I, for one, never thought about how many people would be involved. Thousands of people are now working in a field, genomics, that was not named 12 years ago.

There is a flow of biologic information (table 1) from DNA to RNA to proteins to 3-dimensional structures to assemblies of structures and pathways to interactions with small molecules. This flow is described by words such as *genome*, *gene expression*, *proteome* (the world of proteins), *phenome* (the world of function), and, most important to the private sector, *drugs* and *chemistry*.

The biologic information starts as 1-dimensional, but it becomes 3-dimensional and even 4-dimensional. It is more difficult to deal with things in higher dimensions, so we are going to be more challenged as we move over the next decade from relatively easy 1-dimensional molecules, DNA and RNA, to difficult multidimensional objects, like functional assemblies of proteins.

Our ability to handle biologic information, with today's technology, varies from very poor to very good. If we have small-molecule-binding data to characterize or biologic function to observe (someone has blue eyes or blond hair, and so on), we can interpret readily. For most people, trying to look directly at the information in DNA is like trying to translate hieroglyphics; even at the RNA level, for most of us, trying to make sense out of sequence data

TABLE 1. **The Flow of Biologic Information**

	DNA	RNA	Proteins	Folded Structures	Function: Assemblies Interactions Pathways	Small-Molecule Binding
Catchword	Genome	Gene expression	Proteome	Phenome	Proteome	Drugs, chemistry
Dimension	1-D information	1-D information	1-D information	3-D structure	3-D structure	Time-dependent events
Interpret ability of raw data	"Hieroglyphics"	"Greek"	"Greek"	Picture	Readable	Readable
Complexity	Human 3×10^9 base pairs	100,000 genes	100,000 proteins	2,000 motifs	$>10^{12}$ combinations	$>10^{40}$ small molecules
Species dependence	All different	Most different	Most different	Most same	Most same	Most same
Relative abundance	All genes 1:1	1:10,000 dynamic range	1:100,000 dynamic range	—	—	—

is like trying to read Greek without knowing the language. Many of the DNA data that are most easily obtained—sequence data—are the most difficult to interpret.

A few key numbers characterize the field of genomics. The human genome is the haploid genome; 1 copy is 3×10^9 base pairs of DNA. Current estimates of the number of genes cluster around 100,000. Usually, 1 gene codes for only 1 protein, so there are roughly 100,000 proteins. The motifs mentioned in the previous presentation are the fundamental building blocks of 3-dimensional structures. No one knows how many there are—possibly only a few thousand. When proteins are combined into assemblies and functional pathways are created, the number of such entities can be astronomical. The number of known small molecules is far in excess of 10^6, and the number of potential small molecules is close to unlimited.

As one goes from person to person or from species to species, we are frighteningly different at the DNA-sequence level. However, as one moves toward higher order structures and into the world of function, similarities among various species are much greater. Thus, depending on which viewpoint one takes, the environment is either a difficult problem viewed at the level of DNA sequence or a simpler problem viewed at the level of common biologic function.

Different dynamic ranges are encountered as we progress from the world of DNA to the world of function. We do not know enough to be rigorously quantitative, but DNA, roughly speaking, is monotonous. Most genes are present in a 1-to-1 ratio. However, at the level of gene expression or protein synthesis, dynamic ranges that are fairly formidable appear.

STATUS OF THE GENOME PROJECT

The genome project is doing well. Maps have been made, and sequences have been completed.* The number of completed genome sequences is changing almost daily (table 2). These are all very small genomes. The biology emerging from this knowledge is astounding, but our ability to interpret the sequences is so poor that the number of genes that code for an RNA product, rather than a protein product, is uncertain. As Jurgen Brosius and others have shown, the numbers listed for such genes in table 2 are probably wrong. As a result of the first microbial-genome projects, we are already drowning in important and fascinating information. As more genomes, especially those of microorganisms, are sequenced rapidly over the next few years, the surfeit will get greater and greater.

The results in table 3 are less optimistic. The table shows the progress in completing the sequence of the 6

*At the time of the symposium on which this paper is based, the sequencing of 7 genomes had been completed and the data were available in the public sector.

TABLE 2. **Genomes with Known Nucleic Acid Sequence**

Species	No. DNA molecules	No. kb DNA	Largest DNA	No. ORFs[a]	No. genes for RNA
M. genitalium	1	580	580	470	38
M. pneumonia	1	816	816	677	39
M. jannaschii	3	1,740	1,665	1,738	~45
H. influenza	1	1,830	1,830	1,743	76
Synechoncystis sp.	1	3,573	3,573	3,168	?
E. coli	1	4,639	4,639	4,200	?
S. cerevisiae	16	12,068	1,532	5,885	455

[a]DNA sequences that appear capable of being translated into proteins long enough to be functional.

canonical organisms that were picked by the National Research Council Committee on the Human Genome in the late 1980s. The 2 smallest have been completed, but even 5 years ago there was a considerable amount of sequence information available on these organisms.

Caenorhabditis elegans sequencing is going extremely well, and the estimates are that it will be completed in about another year, but *Drosophila*-, mouse-, and human-sequencing projects have just begun, and the numbers shown in table 3 are hard to come by and hard to stand by. It is difficult to know what is redundant in the sequence databases.

PRIVATE-SECTOR ACTIVITIES

One remarkable thing about the genome project was how aggressively, forcefully, and, I think, effectively the private sector entered the project and in many ways attempted to skim the cream. I would like to say it was predictable. I would like to say we anticipated it, but I think hardly anyone did.

Table 4 shows, as of April 1, 1997, a list of some of the public companies whose sole declared mission is genomics. The aggregate market value of these companies is $2.5 billion. Other companies are not public, so they do not have value that we can easily calculate. At least 1 major additional public company, Affymetrix, should be added to the list; its valuation is surely in the $0.5 billion range. It is easy to do simple-minded calculations on these numbers and conclude that the private-sector effort in genomics is already greater than the government-funded effort worldwide. And it is likely to continue—many of the large pharmaceutical companies are beginning to mount substantial genome efforts of their own.

TABLE 3. **Progress in DNA Sequencing of Chosen Targets**

		Finished Sequence, Mb		
Organism	Complete genome, Mb	June 1992	February 1997	Comment
E. coli	4.6	3.4	4.6	Complete
S. cerevisiae	12.1	4.0	12.1	Complete
C. elegans	100	1.1	63.0	Cosmids
D. melanogaster	165	3.0	4.3	Large contigs[a] only
M. musculus	3,000	8.2	24.0	Total, assuming 2.5× redundant
H. sapiens	3,000	18.0	31.0	In contigs >10 kb
			116.0	Total, assuming 2.5× redundant

[a]Sets of overlapping clones or DNA fragments.

TABLE 4. **Values of Some Genome Companies (April 1, 1997)**

Name	Market Valuation (millions of dollars)
Human genome sciences	673
Incyte pharmaceuticals	559
Millennium pharmaceuticals	332
Genset	322
Myriad genetics	275
Sequana therapeutics	131
Microcide	128
Genome therapeutics	123
Total	2,543

I think it is important, as we go forward, to ask how we should optimize the roles, separate as they are, of the private and public sectors. Who is going to do what? I do not have a simple answer, but I will try to point out places where I think it is critical for the public sector to keep focused on issues that the private sector is not going to focus on, because the scale on which the private sector operates and the speed at which it operates are impressive. That all these companies exist and have raised so much capital means that DNA-sequencing information is viewed as valuable. That is no surprise. Once one assembles a large DNA-sequencing effort, as exists now in both the public and the private sectors, it is a valuable resource. The challenge is to use that resource effectively, and this challenge is sometimes confusing.

Selecting Sequencing Targets

An open reading frame (ORF) is a gene. Some examples of the density of open reading frames are shown in table 5. In *Methanococcus jannaschii,* the archibacterium that is part of the DOE microbial-genome project, there is an extremely high density of genes—1 gene for every 1,000 base pairs. In sequencing this organism, one gets useful information very efficiently. In *E. coli*, the ORF density is more or less the same. In yeast, the density is about 1 gene per 2,000 base pairs. But in the human (we can only estimate, because we do not know the complete sequence), it is 1 gene per 30,000-50,000 base pairs. If one blindly decides to sequence human DNA today, functional units of information will be discovered at only 1/50 to 1/30 the rate for bacteria, if one does simple genomic sequencing.

The challenge is to figure out whether direct human-genome sequencing is worthwhile. Alternatively, given, say, the ability to sequence 100 million base pairs a year, how does one obtain the largest amount of information? I will argue, as devil's advocate, that we should sequence the genes first. We did not know how to do that 10 yr ago when the genome project was started, so it was not part of the plan. But we know how to do it today. If we

TABLE 5. **Density of Open Reading Frames**

Species	Kb/ORF
M. jannaschii	1.001
E. coli	1.105
S. cerevisiae	2.051
Human	30–40 (estimate)

focus on sequencing the genes in the human and many other organisms, we are going to gain important biologic information much faster than if we just sequence the human chromosomes from end to end.

There is another reason to recommend this approach. When the human-genome project was founded, the lie was told that we are going to complete the DNA sequences from telomere to telomere on each chromosome. That is not true. It cannot be done; not with any technology that we have today. Centromeres, as have been elegantly studied by Bob Moyzis and others, are monotonous. There are millions of base pairs of simple repeated sequences with occasional variations. Even if one could figure out how to sequence such regions, most of them are probably unclonable. Doing such monstrous stuff—the last 10% of the genome—might require almost 90% of the total effort, and probably very little could be learned for it.

We should sequence all the genes and then "declare victory" once it is done. As technology gets better and costs go down, we can fill in all the other garbage, which today is not very enlightening.

GENOMICS IN THE FUTURE

I want to turn to the future of genomics. The first issue to address is the design of optimal strategies. The genome project has been tremendously successful thus far. As has been said many times, the project is ahead of schedule and it is under cost. I wish other large science projects were doing as well.

Economies of Scale

A significant reason that the genome project is so successful is that some of its features, such as genomewide mapping versus individual-gene mapping, give an obvious economy of scale. One can argue about the numbers, but huge economies of scale have been gained in some genome-related manipulations. Leroy Hood's presentation described transcript display as an alternative to looking at 1 gene expression at a time. It is obvious that there is an advantage. However, it is not clear that anyone has figured out how to do an equivalently effective display of proteins.

Mutation detection, if one has to do it by direct DNA sequencing, is very inefficient because one has to sequence millions of base pairs to find 1 mutation. If someone can come up with a method of comparative sequencing that projects only the differences, we will gain an enormous economy of scale.

Wherever one can move human-biology problems into simpler organisms—such as yeast or worms, which have short life spans—it affords a large economy of scale. It is not obvious to me that there is any economy of scale in making all possible transgenic mice, a task I am sure people think about. Similarly, in structure determination: At this point, although we have gained speed, it is not obvious yet what the economies of scale are.

One reason DOE deserves enormous praise is that it has focused a considerable fraction of its resources on the development of new and enabling technologies that promise in the future, or even right now, to gain economies of scale. Array technology is something that I knew would be covered in Leroy Hood's presentation, so, in spite of its importance, I will not dwell on how arrays gain an economy of scale because of parallelism.

Another potential new method that could provide an economy of scale is mass spectrometry (MS). It allows both high-speed work and multiplexing; many things can be done at once through multiple labels. Other new technology that seems promising is the microfluidic circuit, which is essentially a laboratory on a chip. This promises both parallelism and speed. The last set of potentially new techniques incorporates the power of intelligently used biology, whether through genetic selections or genetic manipulation. This is one of the ways that we might be able to get some economy of scale in studies of function.

The Challenge of Genetic Testing

Thus far I have presented a very rosy picture, but not everything is rosy. At the beginning of the human-genome project, we promised that by the end of the project we would be able to do "genetic fingerprinting" and, depending on how good the technology became, look at the 100–1,000 most-common genetic diseases, predispositions, or environmental risk factors in a single relatively inexpensive test—everyone could be genetically pro-

filed at birth. That promise looks more remote today. The reason is not that our technology has not improved; in fact, it has improved remarkably. The reason is that the problem of genetic testing has turned out to be much harder than we thought it would be.

The picture of a genetic disease that I think most of us had 10–15 yr ago was hemoglobin S. One base change, 1 allele, caused the same disease in everyone affected. That picture is not representative. Figure 1 shows the result of the last time my co-worker, Joel Graber, peeked into the European Bioinformatics Institute mutation database for p53, a gene important in breast cancer, colon cancer, and probably many other cancers. The figure shows the spectrum of known mutations; the vertical axis is the number of times that a particular mutation has been seen, and the horizontal axis is the position in the gene. The complexity of the mutation spectrum is extraordinarily complicated, and it is typical. If one wanted to do a diagnostic test to guarantee that someone was free of any of these known disease-causing alleles, one would have to sequence the whole gene or come up with a totally new technology that would allow inference that the gene was normal without sequencing it. To make things worse, if we look at the existing data on p53, about 5,000 mutations are known. Almost all of them are single-base substitutions. We can think of fancy tricks to find larger changes easily, but these are not larger changes; they are all single-base substitutions, so methods need to be developed to attack this problem. Today, many of the methods are not user friendly.

Today, mutation detection usually requires accurate DNA sequencing. Now that we know what the mutation

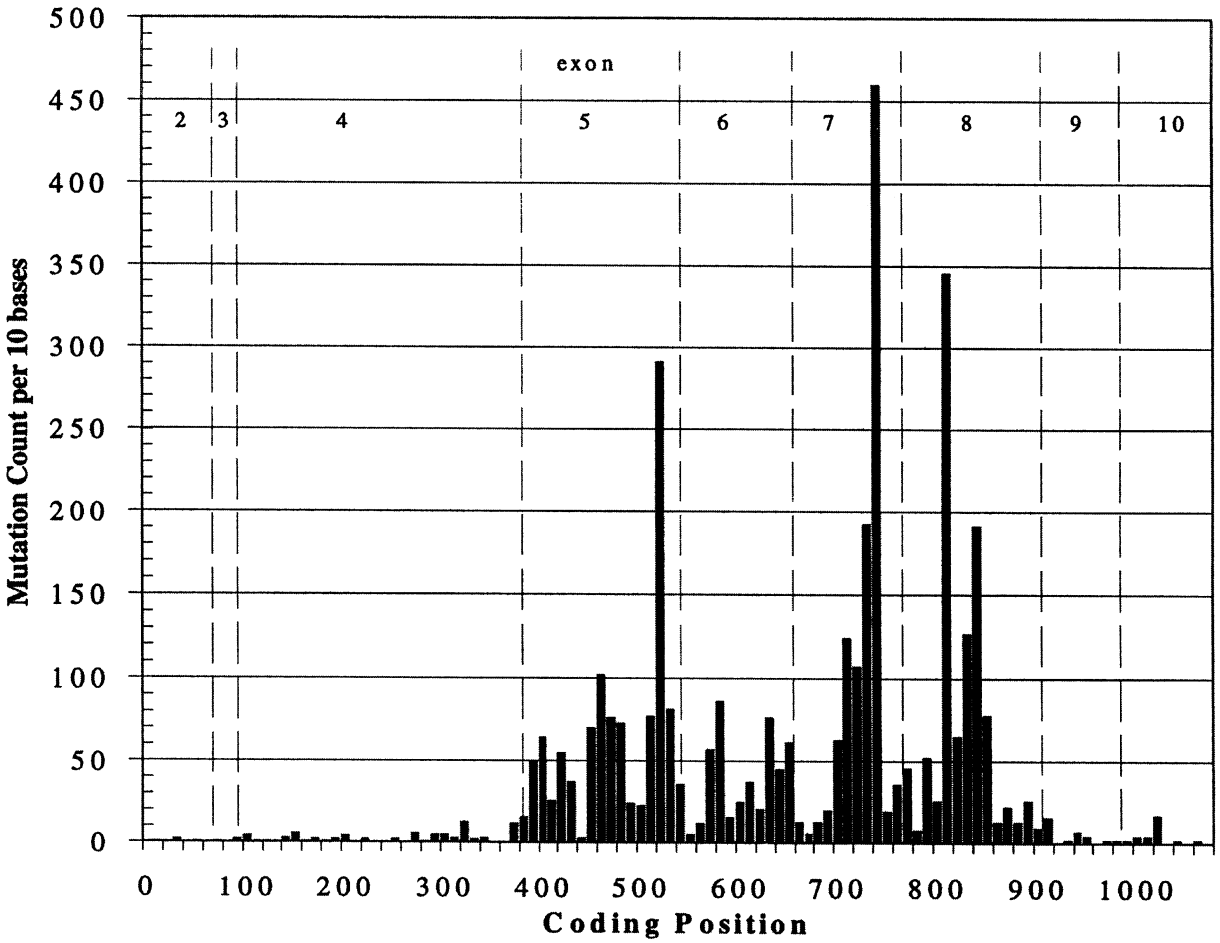

FIGURE 1. Spectrum of mutations seen in p53 expressed sequence.

spectrum is like, the challenge for genetic fingerprinting is the following: Suppose that we are willing to try to score 6 x 10^6 people at birth; this is 0.1% of the total diploid human genome. Such a goal is ambitious, but it is not ridiculous. From just a few big genes like BRCA1 and BRCA2, one might get up to this scale fairly quickly.

A number that always depresses me is that there are 150×10^6 births a year, so to do worldwide genetic screening the annual mutation-detection sequencing requirement is $150 \times 10^6 \times 6 \times 10^6 = 9 \times 10^{14}$ bases, which is a very big number. Dividing by 300 yields the sequencing capacity needed per day, 3×10^{12}. If we had some magical instrument that could do, not 10^5 as today's instruments can do, but 10^9 bases per day, we would need about 3,000 instruments worldwide to satisfy the demand. That is probably equivalent to the number of magnetic resonance imagers worldwide, so it is a reasonable number, but the number that is not feasible with today's technology is this 10^9 bases per day per instrument. Optimized current technology will probably get us another order of magnitude beyond the current 10^5, but for that we need new technology. Array-chip hybridization in principle could go to 10^7 or 10^8 bases per day with proper design.

Those numbers are not preposterous. As *E. coli* synthesizes its DNA, when it replicates it is actually doing so at the rate of 2×10^8 bases per day, and it is reading the sequences accurately. But it is not telling us what the answer is.

The Potential of DNA Mass Spectrometry

We need to develop new methods if we are going to attack problems like human variability and human pharmacogenetics. The current methods will not do it, and I cannot resist using this occasion to mention my favorite among the new methods. DOE has invested a great deal in MS programs, and the effect of this and other investment worldwide is beginning to pay off: In the last year, from being a fantasy, DNA-sequence analysis by MS has become a reality. The method used is called MALDI-MS, *matrix-assisted laser desorption ionization* MS.

The appeals of this particular form of MS are several. The spectra are simple, the instruments are simple, and the measurements are rapid—they take less than 1 s/spectrum. In fact, they could be pushed to 1 ms or maybe even 1 μs if one wanted to.

In MALDI-MS, the sample is combined with an organic crystal, put on a surface, and hit with a laser pulse. The crystal is vaporized, and, it is hoped, the DNA molecule that one is interested in stays intact. It is accelerated by an electric field, hits the target, and is detected.

The impressive thing about the MALDI approach, as opposed to most technologies used by biologists, is that it yields a very-high-resolution measurement. If one measures the time of flight—how long it takes the molecule to get to the target—the resolution for short pieces of DNA is 1 part per thousand. If one puts the ion into a stable orbit, essentially as a tiny synchrotron, and measures what is called ion cyclotron resonance with Fourier-transform MS the resolution is 100 times higher.

The challenge is to figure out how to use these techniques. Although they are powerful, they are limited to relatively short pieces of DNA, and several physical problems, fairly severe, are posed in achieving high resolution as one pushes to longer pieces. Nature has been kind in designing nucleic acids, almost as though she had the mass spectrometer at heart when she chose the bases, in that they differ from one another substantially in mass. The worst case is A versus T, which is 9 daltons (D), but in general different bases are easily distinguishable. A piece of DNA of length L can have L^3 different base compositions, or L^3 different masses, so the possibility of looking at many things at once is very rich.

A 30-base unit of DNA has a mass of 9,000 D. The resolution limit of 1/1,000 of that is 9 D. Therefore, with the possible exception of distinguishing an A from a T, relatively simple MS can distinguish almost everything in 30-base DNA fragments.

When confronted with a potentially novel sequencing method, people always ask, Has anything actually been sequenced with it? I would like to describe a recent pilot project to demonstrate the feasibility of DNA sequencing with MS. The work has been done by a consortium of researchers in Germany and the United States. Robert Cotter at Johns Hopkins; Hubert Koester at the University of Hamburg; and a company named Sequenom, with laboratories both in Germany and in the United States; are involved.

What is done is just ordinary, solid-state sequencing chemistry, but instead of examination of DNA-fragment

lengths with electrophoresis, the sample is mixed with a crystalline organic matrix that is blasted into the vapor phase by laser excitation, and the sequence is read from the masses of the fragments detected. The technique is called MALDI time-of-flight mass spectrometry. The data are getting better and better in our hands and in other people's hands week by week. For example, a run of Gs, which is very difficult to work with in ordinary electrophoretic sequencing, is well resolved with MS.

To demonstrate feasibility, we have sequenced 4 of the exons of p53 with mass spectrometry. We used a walking primary technique, because our read length is short and because we wanted to read through each primer in turn to make sure that the primers were not covering up mutations. The results were sharp peaks and accurate sequencing data.

Those results are encouraging, but they do not mean that MS is ready to replace the thousands of gel-based sequencers in current use. There is a problem in that MALDI-MS is hard to automate. MALDI yields excellent data, but in most conventional MS one has to search around the sample to find what is called a "sweet spot." If one simply hits the sample with a laser at random, no useful data are obtained. A manual search has to be performed, usually under the trained eye of an experienced person looking for the one little place in the sample that gives good MS results.

Recently, our group of collaborators finally solved that problem. We put our samples into wells etched in single-crystal silicon. This resulted in quite reproducible data without the need to hunt. The sample holder is 1 cm^2 piece of silicon with 100 little holes in it. The time is coming when we will be able to analyze DNA very rapidly with MS. However, I chose those words very carefully. I said "analyze," not "sequence," because I do not think that MS is a smart way to sequence DNA. It can do something better than sequence: It can check the sequence by measuring the mass. The peaks seen for DNA fragments with MS are so sharp and so well defined that any variation from the normal shows up as a detectable change in mass.

For most applications where the sequence is nominally known, we can check it; if the sequence is normal, we do not have to determine the sequence. Consider the problem as finding a single mutant base in p53, which contains 1,200 bases. If one has to ask whether each base is normal as one reads through the sequence, it is a waste of time because the answer is yes, the base is normal in perhaps all cases or all but one. With MS, it will be possible to look much more quickly at fragments of a gene and make sure that they are normal; sequencing will not be needed except in the very rare cases in which they are not normal.

I would like to describe 1 final example of DNA analysis with MS. In current human-gene mapping, in forensics, in many other applications of DNA technology, we like to work with the lengths of alternating simple repeating sequences, like ACACACAC. Because these sequences are rather unstable, their lengths vary among different people. Short tandem repeats are 1 of the 2 powerful sets of markers that we have to look at in the human genome and other genomes. These are conventionally analyzed with gel electrophoresis. To make a long story short, gel-electrophoretic patterns are unexpectedly complicated; they are difficult to read, and we now know, because of the MS results with identical samples, that some of the gel-electrophoretic results are artifacts of electrophoresis.

We have seen a number of cases in which the mass spectrum clearly indicates only a single allele (repeat length) and gel electrophoresis, for some reason, shows 2 or more bands. Those cases appear to be common enough to suggest an advantage in moving out of the liquid phase, where DNA secondary structures can cause problems, into the vapor phase, where there are no secondary structure effects. Repeating sequence lengths are used in genetic-linkage studies; a single mistake in a linkage study can be ruinous. Thus, MS appears to offer advantages.

Functional Genomics

I want to end this presentation at the level of gene function because that is really the heart of biology. The use of genome information is going to be extremely complicated, in that it takes us to the level of function, and function is much more difficult than just sequence information.

There are a number of reasons why function is difficult. Sequences that control gene-expression patterns are still a mystery, and we are rather impotent in dealing with them. Proteins are quite difficult compared with nucleic acids. Each protein is an individual; it has idiosyncratic properties. It can be hard to purify. Pathophysiology is

complex and is very species-dependent. The isolation of a human-disease gene does not mean that the same disease phenotype will result when it is put into a mouse. Genes do not work alone. There is a combinatorial problem of immense complexity to deal with in sorting out issues of function.

I will present a brief case history of 1 study of function. In the past, to understand the function of a single gene has required a scientific lifetime of effort. This case history was an attempt to perform a genetic manipulation of the simplest function that I can think of: asking a cell to die on demand. This example arose in the context of environmental control. The work was a collaboration with David Kaplan, who was at the Army Natick Laboratory but is now head of the Biotechnology Center at Tufts University. Our objective was genetic engineering of a microorganism, the soil bacterium *Pseudomonas putida*, so that, when it was released into the environment, it would metabolize aromatic hydrocarbons in a spill until the hydrocarbons were gone and then kill itself. We needed to find a "poison pill" that we could insert into the genome of this organism. The pill would have to be linked into the genetic-control circuitry of the organism, stay silent until the hydrocarbons were gone, and then be activated and kill the organism. Compared with the control networks that Leroy Hood described, this is very simple. We were working in a bacterium—1 cell—and asking it to do 1 thing.

P. putida has a large plasmid under the control of a promoter called $P_{M,}$ which is activated by the product of the *xyl*S gene in the presence of aromatic hydrocarbons, like 3-methyl benzoate; so if the bacterium finds a munitions spill or the equivalent, this pathway is activated, and a set of catabolic enzymes is made that metabolize all those hydrocarbons. For the pill, we decided to use the protein streptavidin, which tightly binds the natural vitamin biotin.

Streptavidin is, in principle, a very effective poison because that is what nature designed it to be. Streptavidin is a natural antibiotic. It is made by the bacterium *Streptomyces avidinii* and secreted in an inactive form by that organism. It diffuses into the environment, becomes activated, and kills everything in the environment because it depletes the environment of biotin. Thus, the streptomyces has a competitive advantage.

The problem that one faces in trying to perform this simple killing function is that, before it is needed for suicide, the streptavidin concentration in these organisms must be effectively zero. If it is not zero, it will make the organism sick; there will then be a tendency for cells with mutations that inactivate the control system to grow preferentially, and the net result is that the pill will be disabled. Once the organism has finished its hydrocarbon meal, the streptavidin molecules are needed for suicide, and they should appear rapidly and kill the host before it has a chance to escape by mutating. Designing a regulating system that will perform in this way is hard.

The 3-dimensional structure of streptavidin was the first novel structure solved by the multiple-wavelength anomalous dispersion method, which is uniquely enabled by cyclotron radiation and makes DOE's synchrotron sources an absolutely essential resource for any kind of modern structural biology.

Streptavidin kills organisms because it interrupts a number of key metabolic pathways, including pyruvate metabolism and fatty acid biosynthesis; bacteria do not have a chance once this material is expressed inside them. The problem is how to control such a lethal poison. Figure 2 shows the simplest model that one can think of. The organism's natural control pathway, 3-methyl benzoate activating the *xyl*S gene, is used to make an inhibitor to keep the streptavidin gene shut off. The cell should survive. When this pathway is turned off, streptavidin expression is no longer inhibited, and the cell should die.

This simple system does not work at all. The control is not nearly tight enough. So we tried a more-complicated system. William Studier, at the Brookhaven National Laboratory, developed what is the gold standard today in bacterial expression systems, a system sufficiently complicated that I will not try to go through all the steps here. It has many more steps than are shown in figure 2. Four processes are connected. The cells survive as long as they are growing on 3-methyl benzoate, but a cascade of events occurs and kills them if they are not growing on 3-methyl benzoate.

We dutifully engineered all of the Studier pieces into *Pseudomonas putida*, and it did not work at all. It is not tight enough. At that point, we were almost ready to throw in the towel. Fortunately, Cassandra Smith, a co-worker on the project, had a clever suggestion: Make the control even tighter by not just operating on the protein level, but also by making antisense RNA. This is a fancy trick; the details would take quite a while to go through. Basically, what we have done is create redundant control pathways. Instead of having 1 pathway for each event, we have 2. This acts not only like a fail-safe system, but also like a quicker system, so that the cells survive as long

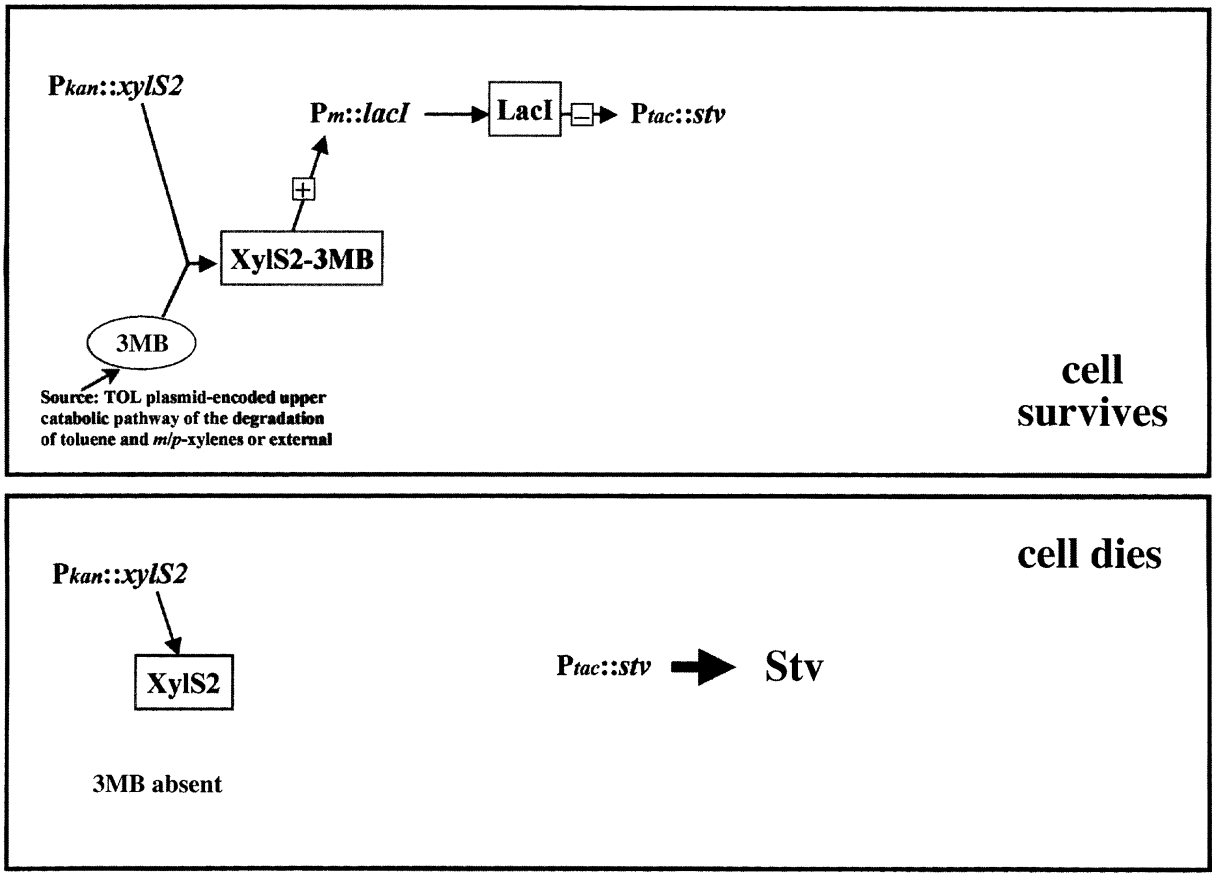

FIGURE 2. Suicide-control system: simplest possible model.

as they are metabolizing 3-methyl benzoate, or anything else like it, but they do kill themselves rapidly once they run out of hydrocarbon.

The actual mechanics are amusing, and the details are sketched in figure 3 for the cognoscenti. One clones the genes that will be expressed out of phase, head-to-head under promoters without terminators. Then, for example, when lacI is expressed, it also makes antisense strands against the streptavidin transcripts. When streptavidin is made, it makes antisense strands against lacI transcripts. By doing this kind of systems engineering, we have made nearly perfect on–off switches, and the system works well.

The killing is better by a factor of 10, better than anyone else has done before in any kind of suicide system. But it is still not good enough for environmental use; we still have some problems with mutagenic escape from suicide. In the next year, we plan to make the system even more complicated. We are going to introduce in parallel an entirely separate second suicide pill; if the organism tries to escape from 1 pill by mutating, it will still be subject to the other. The 2 pills must not have any components in common; if they do, a single mutation could rule them both out. We do not know whether this new system will work well enough to allow anyone to use the organism in the environment. I hope that that vignette provides the flavor of how complex life is, especially when one is dealing with living organisms at the level of function.

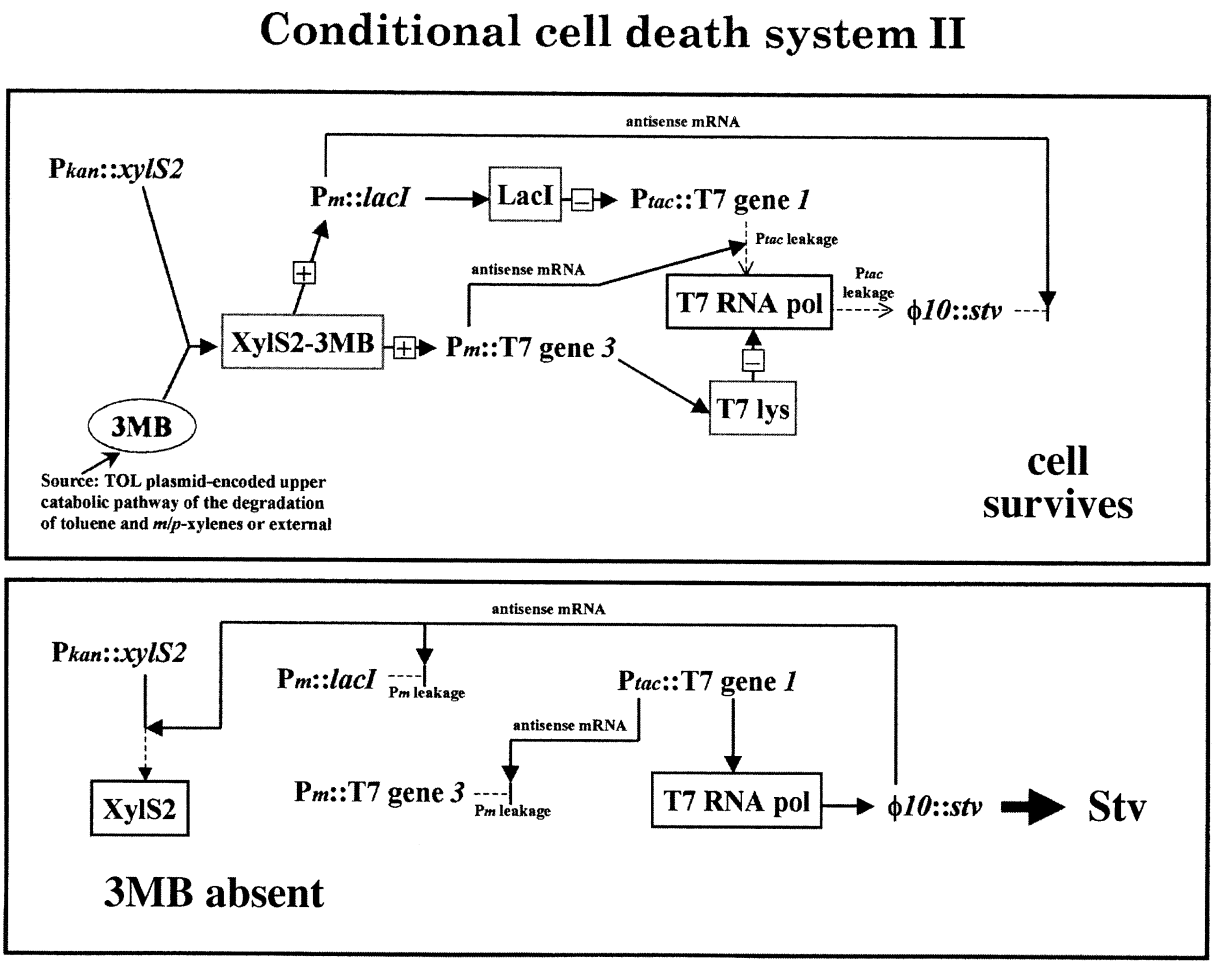

FIGURE 3. Suicide system: construct that was actually functional.

CONCLUSION

My goal here has been to show that we have come a long way very fast. What the genome project has done is open up a tremendous challenge. When the project was conceived, the notion was that we would have 100,000 genes to study in the year 2005, but we have 100,000 genes to study right now, and this is the enormous challenge that presents itself across a wide range of problems from information science through structural biology to plain biology.

Discussant

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In the late 1970s, biologists were beginning to see that the new technology of sequencing DNA was going to affect every area of biology, and Walter Good at the Los Alamos National Laboratory was one of the people who saw that. He also saw that having the sequence data in one place and available electronically for mathematical analysis was going to make a huge difference in how well we could use those data. He began what later became the GenBank database. At that time, it was a pilot project called the Los Alamos Sequence Library[1]; Box 1 is from an early internal report at Los Alamos. The database was about a year old, and there were almost 0.5 million bases of DNA.

Now we get something like 1 million bases of new DNA-sequence data per day. Figure 1 does not show it, but the human sequences continue on the next page for a few lines—there were 10 or 20 human sequences in the database at that time. Now, we have about 50 million bases, and we are expecting another 200 million in the next year.

People often compare the human genome to a blueprint or a map of genetic information—a blueprint for the organism or a map of heredity. But a big difference is that in maps of the earth or in blueprints of buildings, the position of each item matters a lot. In the genome, to a first approximation, the location of a gene in the genome does not matter much. Many genes are independent units—not independent logically, but independent positionally—so in a sense the genome project is a project not to get the sequence, but to get the genes.

GRAIL, a program developed at the Oak Ridge National Laboratory,[2] is the standard tool for finding the genes in DNA-sequence data. We can do a reasonable job of finding the approximate locations of genes. It still requires much experimental work to track down exactly where the genes are and a huge amount of work to find out their functions.

One important method for beginning to understand the function of genes is to see from the DNA sequence, if possible, under what conditions a given gene is expressed, when it is turned on, and when it is turned off. R. Tjian described the initiation complex that begins the transcription of DNA into an RNA copy of the gene.[3] This process is a primary point of control of gene expression. No one knows how many transcription factors—that is, proteins that bind the DNA and control gene expression—there are. There might be tens of thousands. Genes are exquisitely controlled in many different contexts for precise expression just when they are needed.

In a related vein, to follow up on one of Charles Cantor's comments, one thing that computational methods have done fairly well is help us to locate the genes in the DNA sequence. We would also like to be able to locate the precise point labeled the core promoter, where the transcription of the gene begins. We have not done so well on that. There are a number of programs, including some that are fairly obvious and fairly simple. A colleague and I recently finished benchmarking these methods and found, unfortunately, that they do not perform well enough for practical use yet.[4] We have a long way to go.

Biology, however, is a science of special cases, and the right way to solve the general problem of finding the beginnings of all genes might be to find the control points for particular classes of genes. For example, myogenin and mef2 are 2 transcription factors important in turning genes on in skeletal muscle. In the problem of trying to decode these regulatory regions, there is a great opportunity for deep collaboration between the experimental and the information and computational sciences. The interdisciplinary approach not only will pay off, but it is absolutely required. Both those proteins were known to be important in the regulation of muscle-specific genes, and they were known to interact. That is, if you grabbed one with a monoclonal antibody, you found that it dragged the other one along with it. They were known to interact, but the relevant pattern in the DNA—being able to look at the gene sequence and figure out whether the proteins were binding, and so on—was not known.

Figure 1 shows that we can make progress computationally to see what kinds of patterns are important. If you look at the myogenin-binding site in this enhancer, you will see that it is perfectly conserved; the mef2 site is

BOX 1 Directory of Sequences

Two directories to the data bank are kept current and are available on-line. The short directory contains a brief definition of each sequence, usually confined to one line, and so is useful for direct examination. The long directory contains the entire heading for each sequence. The software option DIRECTORY allows it to be searched for the appearance of any character string. There follows first the short directory, then the long one.

LOS ALAMOS SEQUENCE LIBRARY

12/24/81 263 LOCI, 436710 BASES

EUKARYOTIC

BOVGH	BOVINE GROWTH PREHORMONE (PRESOMATOTROPIN) CDNA. 786BP
BOVPOMC	BOVINE PRO-OPIOMELANOCORTIN CDNA. l084BP
BOVPROTHRM	BOVINE PROTHROMBIN 3-PRIME END CDNA. 603BP
BOVPTH	BOVINE PREPROPARATHYROID HORMONE CDNA. 470BP
BOVSAT1706	BOVINE 1.706 G/ML SATELLITE WITH 2350 BP REPEAT LENGTH. 706BP
CHICKATUB	CHICKEN BRAIN ALPHA TUBULIN CDNA. 1364BP
CHICKBTUB	CHICKEN BRAIN BETA TUBULIN CDNA. 1652BP
CHICKHBAS	CHICKEN ALPHA-S-GLOBIN CDNA. 542BP
CHICKHBB	CHICKEN BETA-GLOBIN CDNA. 601BP
CHICKINS	CHICKEN PREPROINSULIN GENE AND FLANKS. 1225BP
CHICKLYZME	CHICKEN EGG WHITE LYSOZYME GENE EXONS AND FLANKS. 680BP.
CHICKOVAL	CHICK OVALBUMIN INCL. FLANKS, 6 IVS. (IVS1,5-7 INCOMPLETE). 4690BP
CHICKX	CHICKEN X GENE 3' END (EXONS 5, 6 & 7). 1266BP
DICTYOM4	DICTYOSTELIUM M4 (LOW ABUNDANCE MRNA) GENE W/TWO IVS. 469BP
DROSAT254	DROSOPHILA 1.688 G/ML SATELLITE REPEATING UNIT. 254BP
DROSAT359	DROSOPHILA 1.688 G/ML SATELLITE REPEATING UNIT. 359BP
DROSO5SR1	DROSOPHILA 5S RRNA GENE CLUSTER REPEAT UNIT. 427BP
DROSOADH	DROSOPHILA ALCOHOL DEHYDROGENASE GENE. 835BP
DROSOMAJHS	DROSOPHILA MAJOR HEAT SHOCK INDUCED PROTEIN GENE & FLANKS. 2832BP
DROSTRNAGC	DROSOPHILA TRNA GENE CLUSTER CODING 8 TRNA GENES. 3224BP
DROSTRNAGU	DROSOPHILA TRNA GENE CLUSTER CODING 5 GLU-TRNA'S (LOCUS 62A). 1684BP
EGCPRIBOP	E. GRACILIS CHLOROPLAST RBSML OPRN 16S-23S SPACER, W 2 TRNA'S. 389BP
FISHINSC	ANGLERFISH PREPROINSULIN CDNA. 655BP
FROG18SRNA	FROG (X.LAEVIS) RIBOSOMAL RNA GENES 18S, 5.8S & 28S(5' END). 2984BP
FROGHBB	FROG BETA-GLOBIN CDNA. 600BP
FROGRRNA	FROG RRNA PRECURSOR GENE- SPACER, INIT&TERM SITES. 1201 BP
FSHSOMATIC	ANGLERFISH ENDOCRINE PANCREATIC SOMATOSTATIN1 PRECURSOR CDNA. 562BP
FSHSOMAT2C	ANGLERFISH ENDOCRINE PANCREATIC SOMATOSTATIN2 PRECURSOR CDNA. 595BP
FSHSOMATC	ANGLERFISH PANCREATIC SOMATOSTATIN PRECURSOR CDNA. 389BP
GOATHBBXPS	GOAT BETA-X-GLOBIN PSEUDOGENE AND FLANKS. 1018BP
HUMAFETO	HUMAN ALPHA-FETOPROTEIN MESSAGE C-DNA. 1578BP
HUMALUHB1	HUMAN ALU FAMILY REPEAT CONTAINING RNA P-ASE III TEMPLATE. 866BP

MEF2 & myogenin: Spacing is Conserved

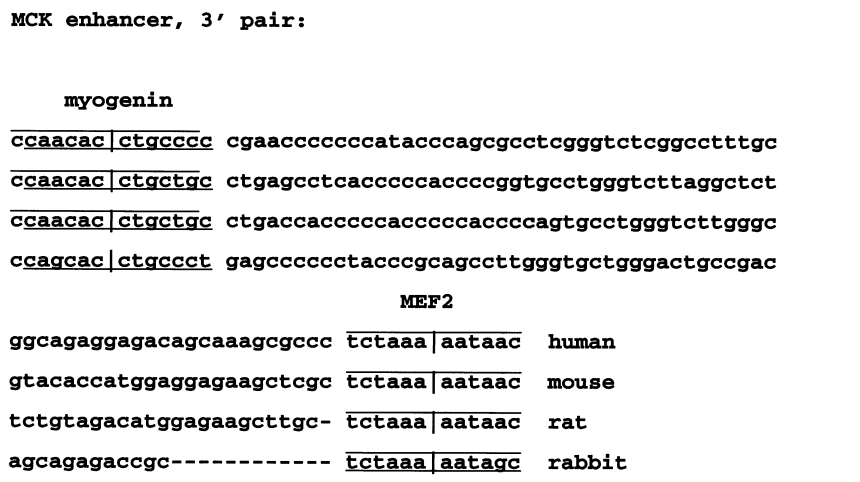

FIGURE 1 mef2 and myogenin: Spacing is conserved.

almost perfectly conserved in these 4 organisms, but in between, the sequence has diverged a great deal in evolutionary history. Nevertheless, the spacing between the sites is perfectly conserved in the first 3 cases, and there is a deletion in the last case that corresponds to 1 full turn of the helix.

There is a geometry here that is clearly important. If you look at the muscle transcriptional regulatory regions in general, you find that this geometry of spacing between the myoD family sites and mef2 family sites is important for muscle specificity and can be used to find some muscle-specific genes.[5] If we combine the experimental studies of finding out where these sites are and the sequence-analysis studies of looking for the geometry of the patterns in the DNA, we will get to the point where we will be able to write computer programs that can look through the DNA and say, Here is a gene that is turned on in liver, here is a gene that is turned on in the atrium in the fetal heart, and so on.

That approach will help in the challenge of finding the function of all the new genes that are turning up in the genome-sequencing projects.

One of the biggest surprises in the genome project has been that, as we have uncovered thousands of new genes, we have found that about half of them do not look like anything we have ever seen before, and we have no clue as to what they do. Clearly, this will require an interdisciplinary approach.

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Discussant

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I would like to speak about one particular component of the Department of Energy (DOE) and Biological and Environmental Research (BER) program: structural biology. As we look toward the future, it is going to be at the disciplinary interfaces where exciting opportunities will develop and revolutionary advances will occur. For structural biology, this will be the interface between chemistry and biology that enables understanding of structure and its function at the molecular level. We have begun to see an evolution in this direction that is driven by a number of other activities, including the genome programs and an increased focus on molecular medicine. As we move into the 21st century, understanding structure and function at the molecular level will become increasingly important, challenging, and rewarding.

I do not have time to talk about what synchrotron radiation is and why it is important; suffice it to say that it is an important enabling technology that provides an extremely intense source of X-rays that can be used to investigate structure. Virtually all we know about the structure of molecules comes from studying the interaction of molecules with electromagnetic radiation. We learn about structure at the level of individual atoms in larger biologic molecules by using wavelengths of light that are comparable with the spacing of the atoms, and that means X-rays. Biologic materials are composed mainly of carbon, hydrogen, and oxygen, so they do not scatter or interact with X-rays strongly. Synchrotron radiation offers intensity increased by a factor of several powers of 10 over conventional X-ray sources (comparable with laser intensities, but in the X-ray region). This has revolutionized our ability to study atomic structure in large molecules with protein crystallography. Synchrotron radiation has also enabled substantial biologic applications of other techniques, such as X-ray absorption spectroscopy and small-angle scattering, which provide information complementary to that obtained with crystallography.

Synchrotron radiation is a polychromatic source, and its multiple wavelengths can be used to solve the phase problem in crystallography (which is necessary to visualize atomic structure). The extremely high intensity of synchrotron radiation allows an even higher level of resolution for biologic molecules.

I would like to discuss how this field has grown and how this has been enabled by the vision of the BER program. What is happening in this field worldwide? It is useful to look at trends in growth for new protein crystal structures. I have talked about X-ray crystallography, but there is also nuclear magnetic resonance (NMR), which is increasingly important for smaller structures. However, NMR has difficulty when the molecular weights get large, as in supermolecular structures, interaction among proteins, and interaction between proteins and DNA or large viruses. X-ray crystallography is an important technology for studying structure in these larger systems. One way of looking at the growth and influence of synchrotron radiation in this field is represented by Carl-Ivar Branden's compilation. He analyzed the number of new structures published in 3 journals—*Nature*, *Science,* and *Structure*—in 1992-1995. In *Nature,* for example, the number of new protein structures based on synchrotron radiation grew from 39% in 1992 to 73% in 1995. Similar growth was seen in the structure papers published in the other 2 journals. That is just one measure of the growth in the importance of synchrotron radiation in solving structures with X-ray crystallography. One finds similar growth trends when looking at the demand for access to the synchrotron facilities.

How has BER played a role in this growth and in meeting demand? In the United States, it supports more than half the funding in this field which provides for the facilities that enable structural biologists to have access to synchrotron radiation. BER funding has grown from a modest level in 1990 to more than $10 million in 1997. The National Institutes of Health (NIH) plays a complementary role in supporting beam lines and operations for structural-biology research at the synchrotrons through its National Center for Research Resources. Together, the support from these 2 agencies is what enables the community to do structural-biology research at these shared multiuser facilities in the United States.

There has been a substantial increase in the use of the 4 DOE-funded synchrotron facilities for structural-biology. The number of users has grown from about 2,000 in 1990 to about 3,000 in 1996, but the fraction of

structural biology or life-science users has grown from only 6-7% to almost 30% in the same period. In part, this is due directly to BER support of activities in the structural biology.

Similar trends are seen in industrial interest in structural molecular biology. There has been strong growth in the number of crystallographers and the number of pharmaceutical and biotechnology companies investing in this technology over the past 5-7 yr, and almost every major pharmaceutical company is investing in the development and support of a structural group. That also translates to increased demand and use of the synchrotron facilities because the same people want access for problems ranging from drug design to structures of modified enzymes with more-favorable industrial properties.

Structural molecular-biology research integrates many parts of the BER portfolio, including relationship to the genome and environmental fields, such as bioremediation. Structural biology cuts across a number of disciplines, components of which transcend many aspects of the portfolio of energy research. It certainly builds strongly on core competences of the DOE. DOE is the nation's premier provider of synchrotrons; the technology, the expertise, and the intellect resides mainly within DOE. DOE operates 4 of the major synchrotron facilities in the United States. It also operates the neutron programs, which I should note are also players in structural biology. Such programs are unique strengths of the national laboratories and provide essential capabilities to the nation's researchers in academic institutions, at the national laboratories, and in industry.

I want to point out 2 more things. One is a recent report from the Institute of Medicine that considered the importance of resource-sharing in biomedical research. The report pointed out the incentives and some of the disincentives for research-sharing. It noted the importance of resource-sharing as one moves toward more and more advanced technologies and pointed out, as a case study, that synchrotron resources are an excellent example.

Finally, *Nature* of May 1, 1997, carried a report by the Biomedical and Biological Sciences Research Council in the United Kingdom. Structural molecular biology was targeted as an important growth field. The analysis concluded that the United States is currently the leader in this field, with more than 50% of the world's output. It also identified synchrotron facilities as essential for the future.

It is a challenge to be a visionary, to think about what will make a serious difference, to see how the BER program can move forward in the best way. Structural biology can be one of the cornerstones on which the future of BER will be built.

Discussant

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The next challenge for the postgenomic era is to determine function from structure. That might sound relatively simple, but it is not, as some examples will show.

Generally speaking, proteins are grouped into families. The families are based on function if that is the information available or on structure if that information is available. Can we put the 2 together? For example, 1 protein family that is well characterized is the globin family, of which myoglobin is a member. The members of this family have similar structures and similar active sites, and those that are involved in chemical transformations catalyze similar reactions.

We might expect to find other families that are unified by structure and function. For instance, the 2 enzymes D-amino acid aminotransferase[1] and branched-chain amino transferase[2] are similar enough at the sequence level that it is expected that their 3-dimensional structures will be similar. That is indeed the case. The 3-dimensional structures look similar, and both enzymes catalyze the same reaction, a transformation called transamination. Ostensibly, if I am looking for a set of transaminases, all I have to do is look for this type of sequence. However, nature is not that simple. Another aminotransferase, L-aspartate aminotransferase,[3] bears no sequence similarity to D-aminotransferase, has no structural similarity to it, and yet catalyzes the same reaction by the same mechanism.

Those 2 enzymes do not resemble each other at all, nor is there any way to derive 1 structure from the other. In addition, it is not possible to predict the function of 1 from that of the other. That is the problem: The structure does not necessarily indicate the function, nor does the function dictate the structure.

A more striking example of the problem is provided by a comparison of 2 enzymes that have similar 3-dimensional structures. Aldose reductase reduces sugar to sorbitol and is implicated in the blindness that is often a complication accompanying diabetes.[4] The second structure has a similar architecture but a completely different function: It is a racemase that is involved in cell-wall biosynthesis.[5] Thus, even if it becomes possible to predict the structure from a sequence, it will be much more difficult, if not impossible, to determine the function.

We need better means of determining structure from sequence and ultimately of deducing function. There have been interesting advances in determining structure from sequence. These are computational and not yet totally accurate, but in some cases at least potentially useful. There are, however, 2 approaches that might be used to determine the function of a protein. They are based on a simple premise: If you can determine an interaction partner for a protein, very often you can determine its function. Consequently, we are going to explore means of determining how ligands interact with proteins. One of these technologies is called solvent mapping, and the other uses combinatorial chemistry. The former method relies on the availability of a 3-dimensional structure; the latter does not.

An enzyme can be represented as a space-filling model in which the protein looks like a solid object. Around this object is a halo of water molecules that covers much of the surface, surmised from biophysical studies and, in part, observed crystallographically. If a ligand is going to interact with this enzyme, it must displace some of the water molecules to reach the surface. In a crystallographic experiment, binding of ligands can be determined directly. However, if the ligand is unknown, then designing the experiment is much more difficult. One method of determining binding sites on the protein and some of the characteristics of the ligand that might associate with such a site is to use small probe molecules to map the surface of the protein. In a series of crystallographic experiments, the surface of the protein can be mapped by a series of organic molecules, representative of parts of a larger ligand. The result is a distribution of such probe molecules over the surface of the protein. These probes tend to cluster. In a series of experiments with elastase and several organic solvents, the probe molecules clustered at the active site in regions indicative of the sites at which side chains of a peptide would interact with the enzyme.[6] This approach is amenable to computational methods, and a number of these have been used to map the surface of a protein by calculating the energy of interaction of a probe molecule with the surface.[6,7]

Thus, determining a structure and the positions of probe molecules, either experimentally or computationally, could make it possible to determine the types of molecules with which a protein interacts, and thereby its function.

A second method of determining function does not rely on 3-dimensional structure. Instead, it relies solely on the ability of a protein to interact with compounds in a library of diverse compounds. With today's synthetic strategies, large variants of a structural motif can be synthesized, and large numbers of structural motifs can be designed. Such libraries are used in the search for unique molecules that will interact with a specific target in the search for new drug leads. Ideally, such a library should cover as much diversity space as possible because the type of interaction that could prove to be advantageous cannot be predicted. In testing such a library against a number of targets, we have found that the interactions observed for any 1 target tend to cluster. For instance, if the targets are a particular receptor, the interactions observed occur with some types of compounds, not others, and not with compounds that interact with, for instance, tyrosine kinases. This result implies that a library of the proper diversity can be used to differentiate types of functions by looking at the types of interactions that an unknown protein undergoes. A relatively simple screening mechanism would let us determine the functions of proteins about which we know nothing.

In summary, 2 types of technologies could be useful in linking sequence to function. One depends on structural, the other on screening, methods. Both require some protein to work with. Obtaining structures of all proteins is not a practical goal. However, the structural method can be applied to proteins that do not yield to other methods. In addition, as structure-prediction methods improve, the method can be used with computers. Screening methods also require protein, and obtaining sufficient quantities is an in vitro challenge. Ultimately, refinements of these methods will be required if we are to turn sequence information into function information about the many gene sequences becoming available.

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Part II

Biotechnology for a Healthy Citizenry

Biologic Imaging: From Mouse Genome to Human Disease

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A partnership of Congress and the Atomic Energy Commission in the Atoms for Peace program created a peaceful and important use for radioactivity and created the field of nuclear medicine. Nuclear medicine now provides about 13 million clinical procedures per year and is an important part of the biologic research programs of not only the United States but the world. My task is going to be to focus on molecular nuclear medicine and help to bring nuclear medicine into a partnership with modern biology and genetics.

My colleagues and I are going to focus on using the power of imaging to look at the biologic basis of organ function in living organisms. Imaging has enormous power. As scientists, we seek to assemble pieces of a puzzle and to put together enough pieces and lines of evidence to show the whole picture of a process being studied. Imaging seeks to create a picture from the very beginning.

The Department of Energy (DOE) is playing a critical role in developing a segment of imaging technologies called biologic imaging. Biologic imaging covers many domains. It combines biology, modern genetics, and the principles of imaging to enable us to look at a whole system—a molecule, a cell, or a whole organism.

Now, why take an image? Medically, we take an image because we want to find out what is causing a problem. Another reason is to see what happens to a drug after a patient swallows it or we intravenously inject it. Imaging lets us watch a drug be distributed throughout a body and understand both how it interacts with its target and how it interacts with the rest of the body.

How do we get images? We use a trace amount of unusual radionuclides—positron-emitting radionuclides of carbon, nitrogen, oxygen, and fluorine (as a substitute for hydrogen or hydroxyl groups). We can use a simple molecule, glucose, and a particular form of it, 2-dioxyglucose, in which a fluorine substitutes for a hydroxyl group. This molecule was created at Brookhaven National Laboratory, developed as a drug at Washington University and then as a tracer for autoradiography.

In our technique of PET, the presence of the positron-emitting radionuclide fluorine-18 on the molecule will tell us where the molecule is after we administer it to a patient. The positron combines with an electron, and then the massive electron and the positron are annihilated, producing back-to-back photons that are easily transmitted through the body and detected by a camera, which is called a PET scanner. The PET scanner consists of a circumferential array of detectors that can detect the photons. This technology was developed with DOE support; when the original system was developed at Washington University, there were 2 sources of financial support: DOE and the Women's Advertising Association of St. Louis.

I am going to talk about 2 studies that used different PET techniques. The first study used glucose metabolism to discern changes in the energy requirements or work of the brain when normal people performed various tasks.

We asked them to open their eyes and look at a scene, which activated the visual cortex; then, we asked them to listen to the mystery story, "The Shadow." They also performed a simple motor task: they touched their thumb to their finger in a forward and rear direction. Some years ago, Jennifer Jones and I appeared before Congress and asked for $8.7 billion. At the end of her presentation, I showed a slide to remind Congress that we now have technologies to determine whether they were watching what Jennifer showed them and listening to what she said, whether they thought about it, whether they remembered it, and whether they would do anything about it. They did give the money.

The other study, at Washington University, used a different approach. We required a subject to perform the task for extended periods—20 or 30 min. A subject could perform a measurement and the task in 30 s. We could map in a single person all the things that we had mapped in multiple subjects. People looked at words, and activated the visual cortex. Then they spoke the words—a motor task that activated the motor cortex. They thought about the words, funnel cortex. This all led to what is called brain mapping, with not only PET, but MRI. We can actually watch a person perform tasks and watch the brain deal with them. We can watch the brain anticipate a task, receive information, sort through it and understand it, and execute a response.

A study of Huntington's disease started an unusual relationship. It was initiated by me and Dave Kuhl at UCLA. Huntington's disease is the textbook genetic disorder. It appears to have originated from one person, a sailor, and is a dominant hereditary disease of total penetration expressed in midlife. We began to look at people who were symptomatic for Huntington's disease with PET. Then we started to look at their children to see whether we could identify who carried the gene before having any symptoms. At the same time, Jim Gazella at Harvard began to use markers to try to find the gene. Gene hunters were looking for the gene, and we were using imaging to wander through the body to look for where the gene was expressed and what it did to organ systems.

We showed in asymptomatic children that we could identify metabolic abnormalities. The Huntington's patient has a severe metabolic deficiency. We could identify changes 7 yr before people ever had symptoms. Gazella finally identified the marker: the Huntington's gene was on the short arm of chromosome 4, and it was called the G8 probe (for Gazella's eighth try). He was lucky the first time. It took a lot of time after that to identify the gene, which has now been done.

Strangely, when we initially did this, about 30% of the patients were discordant; that is, our answer differed from Gazella's and the marker. People said that the metabolic mapping with PET must be wrong. Fortunately or unfortunately, we agreed with the geneticist Mendel. Our prediction agreed with Mendel, and Gazelle's did not. As it turned out, the initial marker was not very accurate. As its accuracy improved, there was complete concordance between PET and the genetic marker.

The next entry was in Alzheimer's disease. We began a project with Duke University in familial Alzheimer's. In Alzheimer's disease, the traditional imaging studies all produce normal results. But there was a hallmark of Alzheimer's disease with PET: a bilateral metabolic deficiency occurred in the parietal cortex and spread throughout the cortex, and we could watch it develop over time. We began a program in which people were examined to determine whether they carried the APOE4 protein or the gene that expressed that protein. These were people who had Alzheimer's in their families, but no symptoms, that is, no short-term memory deficits or cognitive decline at the time of the study. Patients who carried APOE4, which increases the risk of Alzheimer's disease, had the metabolic abnormality characteristic of Alzheimer's. People who did not carry APOE4 did not have the abnormality. We can probably see the expression of the gene developing in Alzheimer's patients 5 yr before they become symptomatic.

How can such things happen? Our organ systems have compensatory actions and reactions to disease. As a gene defect or any disease defect begins, the body begins to compensate. Parkinson's disease patients lose 70-90% of their dopamine before even the most minor symptoms can be identified. A biologic marker can identify a developing disease not only in the earliest symptomatic phases, but long before the disease produces alterations in organ function or loss of regulated function. For example, it is thought that breast cancer begins about 9 yr before we pick it up.

Discussant

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Positron emission tomography (PET) allows the measurement of regional concentrations of positron-emitting isotopes. There are positron emitters for the natural elements of life (table 1), so compounds can be labeled without affecting their pharmacologic behavior.[1] The noninvasiveness of PET provides a unique tool for drug investigations in humans. Until recently, the distribution of psychoactive drugs in the brain, their binding properties, and their effects on neurotransmitters have been investigated in laboratory animals or postmortem in humans. Pharmacologic studies in living humans have been limited to the assessment of a drug's behavioral effects or to the assessment of drug metabolism, concentration, and clearance in body fluids. With PET, it has become feasible to investigate the temporal course of the behavior of the drug (pharmacokinetics) and the mechanisms of action of the drug (pharmacodynamics) directly in the human brain. The short half-life of the positron emitters used with PET (table 1) enables repeated studies, to assess multiple biochemical characteristics affected by a drug, or longitudinal studies in a given subject. Various experimental strategies can be used with PET for the investigation of the effects of drugs, including labeling of the drug itself, neurochemical measurements, and functional measurements. This paper illustrates the power of PET in the investigation of drugs of abuse. Similar strategies can be applied to investigate therapeutic drugs.

LABELED DRUGS TO VISUALIZE DRUG DISTRIBUTION AND PHARMACOKINETICS

Several drugs of abuse have been labeled with positron emitters, usually carbon-11, and investigated in the human brain (table 2). That has enabled quantification of the (percentage) uptake of a drug in the brain, its regional distribution, its binding profile, and its pharmacokinetics. PET can also be used to assess the binding and pharmacokinetics of drugs in other organs.

The use of labeled drugs and PET in drug research is illustrated by the case of cocaine, which is considered to be among the most reinforcing of the drugs of abuse.[2] The reinforcing effects of cocaine have been associated with its ability to block the dopamine transporter (DAT).[3] Blockade of DAT by cocaine results in accumulation of dopamine (DA) in the synapse, which enhances the magnitude of the DA signal (figure 1). One unresolved question regarding cocaine like drugs is why there are compounds that block DAT with similar or higher affinity for DAT but are not reinforcing, as is the case for mazindol.[4,5] Another of these drugs, methylphenidate (Ritalin), has an affinity similar to that of cocaine for DAT. Although methylphenidate has been shown to have reinforcing

TABLE 1 Positron Emitters Most Commonly Used for PET Studies

Radioisotope	Half-Life, min	Decay
Carbon-11	20.4	β^+ (0.960 MeV)
Fluorine-18	110	β^+ (0.635 MeV)
Oxygen-15	2	β^+ (1.73 MeV)
Nitrogen-13	10	β^+ (1.19 MeV)

TABLE 2 Drugs of Abuse Labeled with Positron Emitters and Imaged with PET

Drug class	Labeled drug
Psychostimulant	[^{11}C]Cocaine,[11] [^{11}C]methylphenidate[13]
Sedative hypnotic	[^{11}C]Flumazenil[57]
Opiate	[^{11}C]Morphine, [^{11}C]heroin, [^{11}C]codeine,[58] [^{11}C]buprenorphine[59]
Nicotine	[^{11}C]Nicotine[60]

effects when administered intravenously[6] it is abused much less frequently than is cocaine.[7] One question that arises is whether methylphenidate, the most frequently prescribed psychoactive drug in children in the United States, where it is used for the treatment of attention-deficit disorder,[8] is as potentially addictive as cocaine or whether variables other than affinity for DAT influence the reinforcing effects. Of particular interest is whether these 2 drugs differ in their pharmacokinetics, inasmuch as previous studies showed that the shorter the interval between intake and perceived effects of a drug, the greater its reinforcing effects.[9,10]

To address that issue, we compared the distribution and pharmacokinetics of cocaine and methylphenidate in the human brain by labeling cocaine[11] and methylphenidate[12] with ^{11}C and then measuring their concentrations in the brain with PET.[13] [^{11}C]Cocaine and [^{11}C]methylphenidate were almost identical in this distribution in the brain; both concentrated in the basal ganglia, the brain region with the highest DAT concentration. Furthermore, by performing pharmacologic experiments in baboons, we determined that both drugs bound to DAT[14,15] and that they bound either to the same DAT site or to an overlapping site, in that pretreatment with a pharmacologic dose of either drug was able to block the binding.

PET allows the measurement of not only the distribution of a drug in the brain but also the temporal course of the concentration of the drug in various brain regions, so it was possible to compare the pharmacokinetics of cocaine and methylphenidate directly. The time course for the distribution of [^{11}C]cocaine and of [^{11}C]methylphenidate at the level of the basal ganglia is shown in figure 2. Cocaine goes into the brain very rapidly, achieving peak concentration in 4–8 min, and it also clears from the brain rapidly; after 30 min, there is almost no activity. Methylphenidate, like cocaine, goes into the brain very rapidly, achieving peak concentration in 8–10 min, but its clearance from the brain is much slower than is that of cocaine; at 90 min, there is still a substantial amount of methylphenidate in the brain.

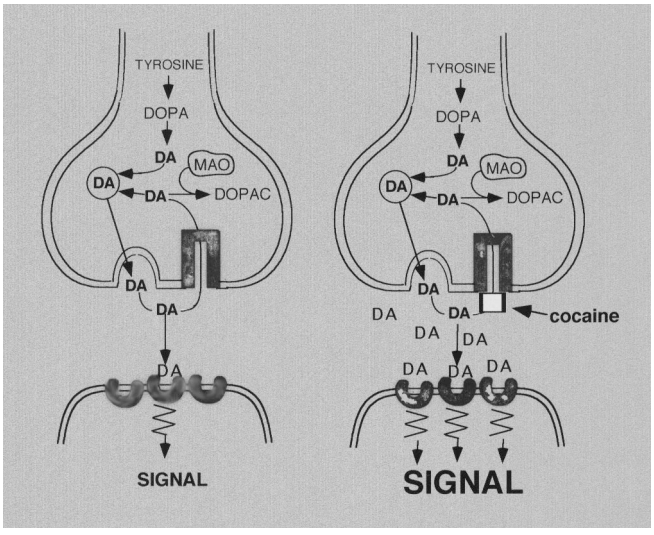

FIGURE 1 Effects of cocaine on DA synapse.

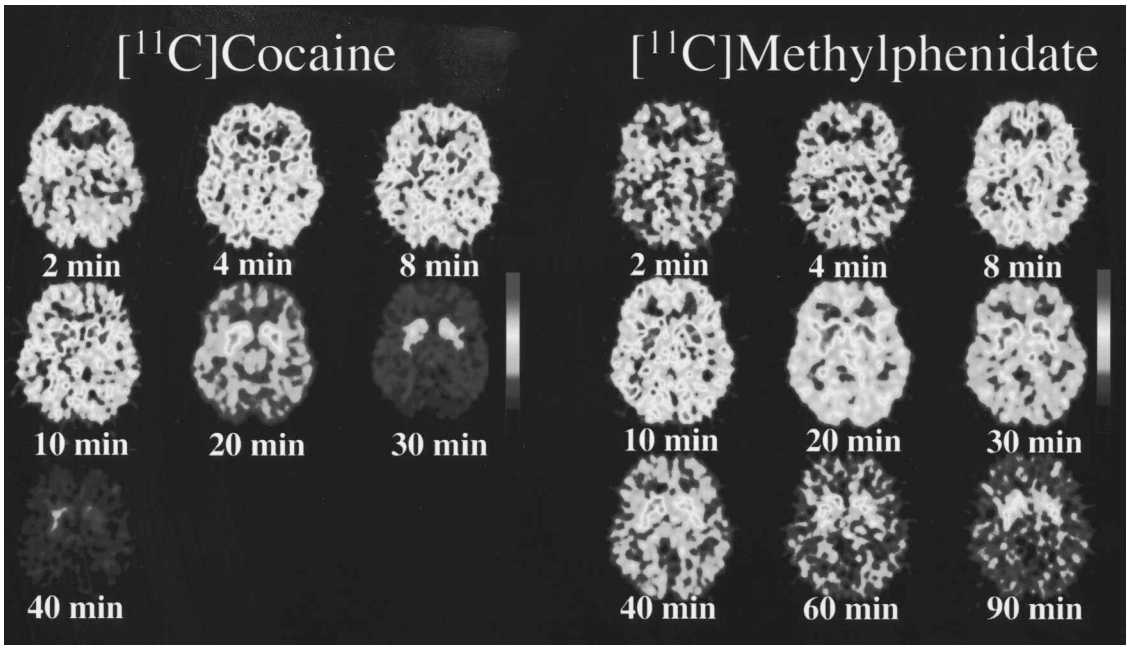

FIGURE 2 PET images obtained with [^{11}C]cocaine and with [^{11}C]methylphenidate shown for 1 plane centered at basal ganglia at different times after administration of radiotracer. Note faster clearance of [^{11}C]cocaine than of [^{11}C]methylphenidate.

Because PET-imaging studies can be done in human subjects, one can simultaneously measure the pharmacokinetics of the drug in the brain and the temporal course for "self reports" of drug effects. That permits assessment of the relationship between the 2 measures. Figure 3 shows the relationship between the kinetics of [^{11}C]cocaine and of [^{11}C]methylphenidate in the basal ganglia and the time course for self-reports of a "high," which is among

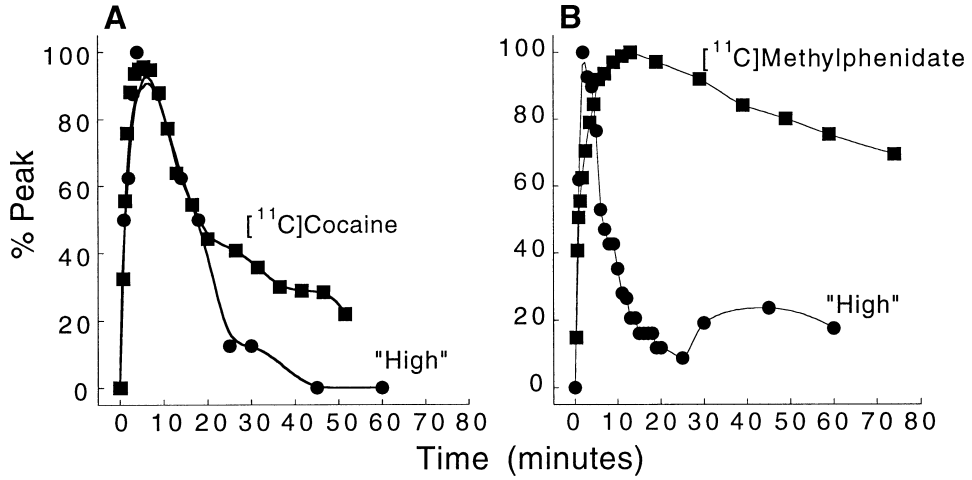

FIGURE 3 (A) Time–activity curve for [^{11}C]cocaine in basal ganglia and temporal course for self-reported "high" induced by intravenous cocaine (0.6 mg/kg iv). (B) Time–activity curve for [^{11}C]methylphenidate in basal ganglia and temporal course for "high" induced by intravenous methylphenidate (0.5 mg/kg iv). Adapted with permission from Ref. 13.

the best predictors for drug self-administration in humans.[16] In the case of cocaine, both the fast uptake of the drug in the brain and the fast clearance parallel the time course of the short-lasting "high." In the case of methylphenidate, the initial perception of the "high" induced by its intravenous administration follows the fast uptake of [^{11}C]methylphenidate in the brain. As for cocaine, the methylphenidate-induced "high" is very short lasting, and it returns to values close to baseline 30 min after administration, despite the drug's remaining in the brain for a much longer period. Thus, the "high" induced by the intravenous administration of cocaine or of methylphenidate appears to be related to the fast uptake of the drug in the brain but not to its continuous presence there.

We postulate that the potential for a drug to induce addiction is due not only to its reinforcing effects but also to its ability to facilitate repeated administration. In the case of cocaine, fast uptake and clearance from brain, which correspond well with the short duration of the "high," promote repeated administration, which is characteristic of the binge behavior observed in cocaine addicts, who repeat the administration of cocaine every 30–40 min.[17] In the case of methylphenidate, we postulate that, although fast uptake evokes the response of a "high" similar to cocaine's, slow clearance does not promote frequent repeated administration. If methylphenidate were taken repeatedly at the time that the "high" returns to baseline, it would rapidly lead to DAT saturation. On the basis of these imaging studies, it appears that it is not sufficient for a drug to block DAT rapidly, but that to enable repeated administration it has to clear from the brain rapidly. Therefore, one can postulate that one reason why cocaine is more reinforcing and more addictive than other drugs that block DAT is its fast pharmacokinetics.

NEUROCHEMICAL MEASUREMENTS

PET can be used to assess the effects of a drug on a neurotransmitter system, including its efficacy at its molecular target and its effects on neurotransmitter concentration.

Drug Efficacy

PET has been used to measure the efficacy of drugs of abuse in producing a specific biochemical effect. For example, in the case of cocaine, studies were conducted to quantify its ability to block DAT in the human brain and to determine the extent of blockade required for cocaine to be perceived as reinforcing.[18] The studies used [^{11}C]cocaine as a radiotracer for DAT. Subjects were repeatedly scanned after administration of a placebo and of different pharmacologic doses of intravenous cocaine. Pharmacologic doses of cocaine compete with [^{11}C]cocaine for binding to DAT in proportion to the number of DATs that they occupy. By comparing the difference between [^{11}C]cocaine binding obtained after administration of the placebo and that obtained after administration of pharmacologic doses of cocaine, one can calculate DAT occupancies with appropriate mathematical models. Figure 4 shows images obtained with [^{11}C]cocaine after administration of a placebo and after administration of cocaine at 0.1 mg/kg and 0.3 mg/kg. Even the 0.1-mg/kg dose, which several of the cocaine abusers did not recognize as different from the placebo, substantially inhibited [^{11}C]cocaine binding in striatum. Figure 5 shows the extent of DAT occupancy for 4 doses of intravenous cocaine—2 (0.05 and 0.1 mg/kg) that are not perceived as reinforcing and 2 (0.3 and 0.6 mg/kg) that are often used by cocaine abusers. For cocaine to be perceived as reinforcing, about 60% of DAT needed to be blocked. These results showed that cocaine is highly effective in inducing DAT blockade. In this study, it was also shown that the intensity of cocaine-induced "high" was significantly correlated with the extent of DAT blockade and that a substantial proportion of DAT had to be blocked for cocaine to induce a "high" (figure 6).

Chronic Effects

To understand the actions of drugs, it is important to recognize that the responses to drugs can vary among individuals presumably as a result of differences in biochemical characteristics of their brains. That is relevant in the case of addiction because the chronic administration of drugs of abuse can lead to biochemical changes that affect later responses to the drugs. The response of the brain of an addicted subject to the drug causing the addiction is likely to be different from the response of the brain of a nonaddicted subject. It is relevant to

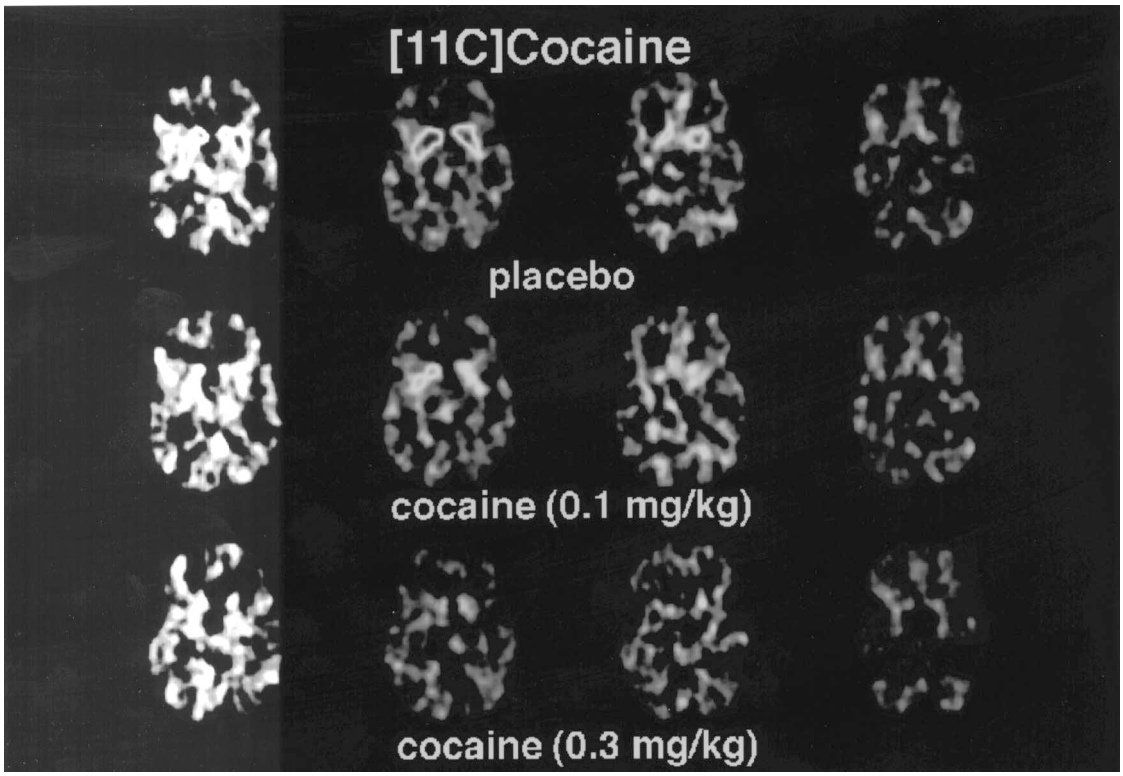

FIGURE 4 Images obtained with [^{11}C]cocaine for 4 consecutive planes at basal ganglia after administration of placebo or intravenous cocaine at doses of 0.1 mg/kg and 0.3 mg/kg. Notice marked reduction in binding of [^{11}C]cocaine when pharmacologic doses of cocaine are coadministered with radiotracer.

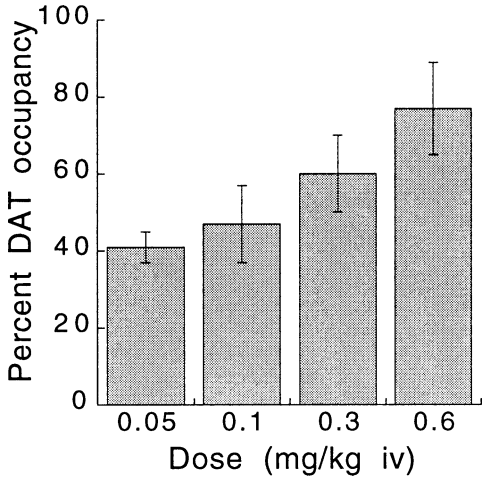

FIGURE 5 Extent of DAT occupancy induced by different doses of intravenous cocaine. Values are means and standard deviations.

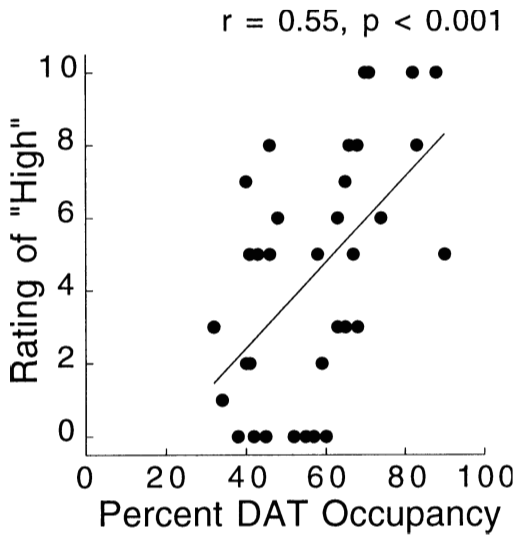

FIGURE 6 Correlation between DAT occupancy induced by cocaine and subjects' self-reported "high." Adapted with permission from Ref. 18.

investigate the biochemical changes induced by the chronic administration of drugs of abuse, because these changes are going to affect responses to the drug and might help one to understand the biochemical changes that underlie the process of addiction. A main neurotransmitter target in addiction is the DA system; increase in extracellular DA in the nucleus accumbens, the brain structure most associated with drug reinforcement, is crucial to the reinforcing effects of most drugs of abuse.[19,20] With PET, one can assess various components of the DA system—receptors, transporters, and synthetic and catabolic enzymes.[21] Studies evaluating the effects of drugs of abuse on the DA system have shown that chronic use of cocaine leads to long-lasting decreases in DA D2 receptors.[22,23] Similar decrements in DA D2 receptors have also been documented in heroin addicts[24] and in alcoholics.[25] Studies of the effects of chronic cocaine use on DAT have been less consistent: Some studies report increases;[26] others show no changes.[27]

Studies measuring the effects of drugs of abuse on enzymes have focused mainly on enzymes responsible for DA synthesis (dopa decarboxylase)[28] and dopamine metabolism (monoamine oxidase [MAO] A and B[29] and catechol-*o*-methyltransferase).[30] Studies using [18F] to measure the rate of synthesis of DA have shown significant reductions in cocaine abusers compared with controls .[31] Studies using [^{11}C]deprenyl-D2 and [^{11}C]clorgyline as radioligands to measure the concentration of MAO A and B have been conducted only in cigarette smokers.[32,33] These studies showed that the concentration of MAO A and B was significantly lower in the brains of cigarette smokers than in the brains of nonsmoking subjects (see figure 7 for MAO B). Because subjects in these studies were asked to refrain from smoking for at least 4 h before being examined, the inhibition of MAO B cannot be accounted for by the presence of the drug itself. Figure 8 summarizes the data on brain concentration of MAO B in 8 nonsmokers (as controls), in 8 active smokers, and in 4 former smokers. Smokers had MAO A and B concentrations which averaged 30% and 40% lower than those of nonsmokers and former smokers. The effects are not due to a genetic difference between smokers and nonsmokers, inasmuch as they reverse once smoking is terminated. Inhibition of 1 main enzyme that metabolizes DA in cigarette smokers is likely to result in a higher concentration of DA at the DA terminal. Thus, one could postulate that the inhibition of MAO in cigarette smokers would raise the increases in DA concentration induced by drugs, including nicotine. That, in turn, could increase the reinforcing effects of drugs of abuse, in that it is the increase in DA concentration in the nucleus accumbens that is associated with the reinforcing effects of most drugs of abuse.[20] Those findings could explain why people addicted to other types of drugs almost universally smoke.[34]

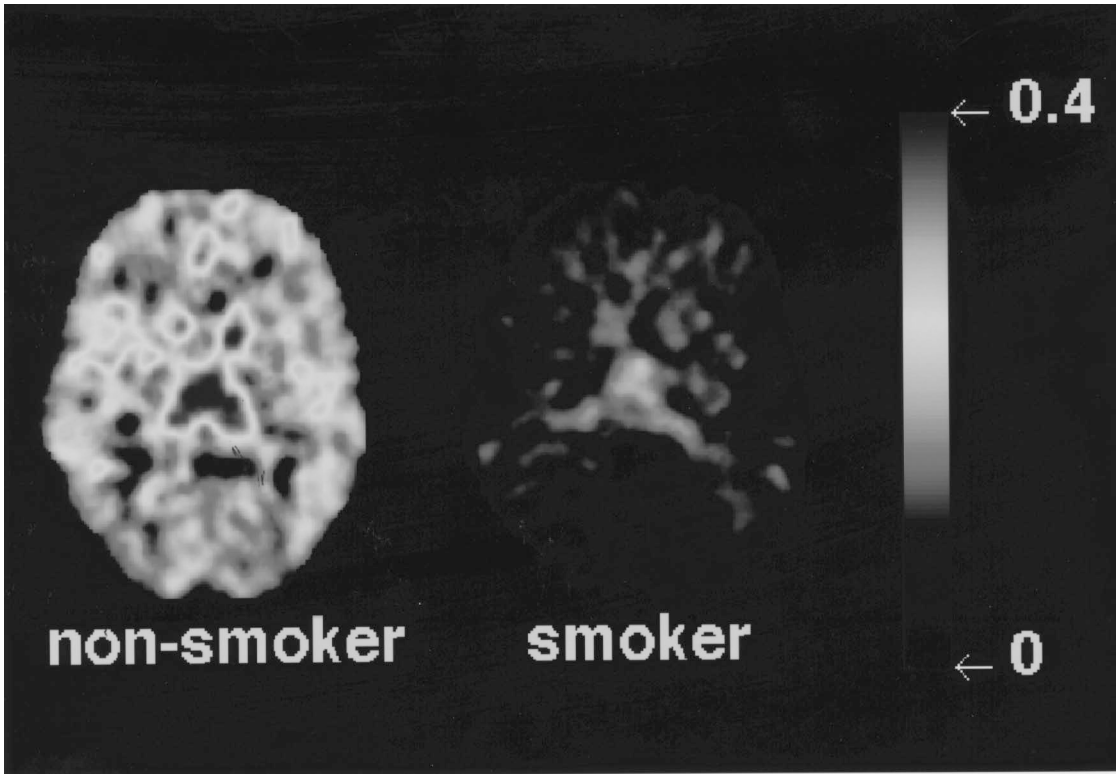

FIGURE 7 Images obtained with [^{11}C]deprenyl-D2, tracer used to image concentration of MAO B, for 1 plane at basal ganglia in a healthy subject and in an active cigarette smoker.

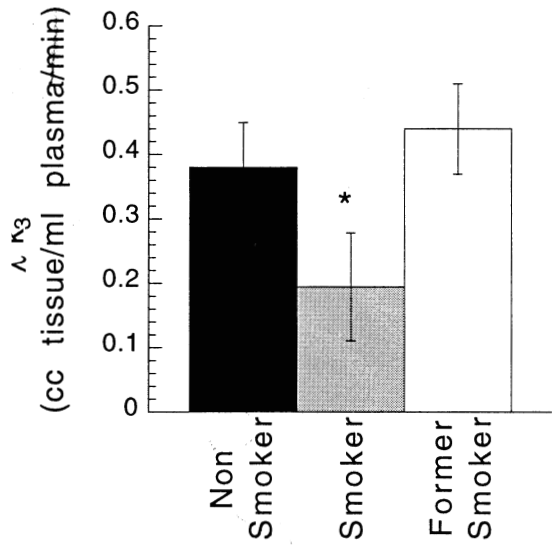

FIGURE 8 Brain concentration of MAO B, expressed as model parameter λk_3, in 8 nonsmokers (as controls), 8 active cigarette smokers, and 4 former cigarette smokers. * $p < 0.0001$.

Neurotransmitter Release

It is possible to assess changes in intrasynaptic DA with PET by using DA D2 receptor ligands, such as [^{11}C]raclopride, which have a relatively low affinity for DA D2 receptors, so that they compete with endogenous DA for binding to the receptor site.[35,36] Drugs that increase DA concentration decrease the specific binding of raclopride, whereas drugs that deplete DA increase its binding.[36,37,38] Furthermore, with a striatal-slice preparation, a very good correlation had been found between the frequency of electric stimulation and the displacement of [^{3}H]raclopride from its binding sites; these effects are prevented by DA depletion with pharmacologic agents.[39] The competition of endogenous DA with raclopride presented the opportunity to measure changes in synaptic DA in vivo with PET by observing the degree of reduction in the binding of [^{11}C]raclopride by pharmacologic agents.[40,41,42] In fact, the magnitude of the changes in DA concentrations assessed with microdialysis in the nonhuman-primate brain has been shown to correlate well with changes in [^{11}C]raclopride binding simultaneously recorded in the same animals with PET.[43] PET studies in humans have been done with [^{11}C]raclopride to assess the effects of reinforcing drugs on synaptic DA concentration.[44,45] For those studies, subjects were scanned twice—at baseline and after administration of the drugs that are known to increase DA synaptic concentration directly or indirectly (figure 9). Studies in normal controls have shown that the magnitude of the change in [^{11}C]raclopride binding elicited by methylphenidate decreases as a function of age.[44] Similar studies in cocaine abusers showed that the magnitude of methylphenidate-induced reduction in [^{11}C]raclopride binding was half that observed in age-matched controls.[46] Cocaine addicts also showed a blunted response to the methylphenidate-induced "high" compared with nonaddicted subjects. The blunting is likely to reflect decreased DA brain function in cocaine addicts. We cannot rule out the possibility that the blunting preceded the drug use in the cocaine addicts, but the findings illustrate how drug history can affect a person's response to a psychoactive drug.

FUNCTIONAL MEASUREMENTS

Regional brain function can be monitored with PET by measuring energy metabolism or by measuring cerebral blood flow (CBF). Brain metabolic rates for glucose and for oxygen are measured with 2-deoxy-2[^{18}F]fluoro-d-glucose (FDG) and with oxygen-15-labeled oxygen, respectively. The most frequently used PET radiotracer for CBF measurements is ^{15}O-labeled water.[47] Because under normal conditions energy metabolism and CBF are tightly coupled with brain activity, the above tracers can be used to measure regional brain function.[48] An advantage of FDG is that it reflects glucose metabolism, which is the main source of energy for the brain; a disadvantage is that it reflects metabolic activity over a 30-min period, so rapid processes might not necessarily be recorded. The main advantage of ^{15}O-water is the feasibility of performing repeated studies in the same person within a relatively short period because of the short half-life of ^{15}O (120 s). A disadvantage of measuring CBF is that many psychoactive drugs have vasoactive properties in addition to their direct effects on nervous tissue; this makes it difficult to separate the direct effects of the drug on the brain from its vasoactive effects.

Functional tracers can be used to investigate the effects of both acute and chronic drug administration and so enable a determination of which brain regions are the most sensitive to the effects of a given drug. With that information, one can predict which neurotransmitters are involved in producing the effects of the drug. Also, because the drug is given to subjects who are alert at the time of the study, associations can be made between the behavioral effects of the drug and its regional brain-metabolic consequences. Although the use of functional tracers to assess the effects of drugs might not be as precise in localizing the site of action of a drug as is the use of more-specific tracers, it nonetheless provides a measure that reflects the final consequences of the effects of the drug in the human brain. That is important because, even though the effects of a drug might involve a specific interaction with a receptor site, the secondary consequences of that interaction might be the ones relevant for its pharmacologic effects.

Brain Metabolism

A PET study of the effects of 2 sequential doses of methylphenidate on brain glucose metabolism, measured with FDG in normal controls, exemplifies the strategy. Two intravenous doses of methylphenidate were given 120

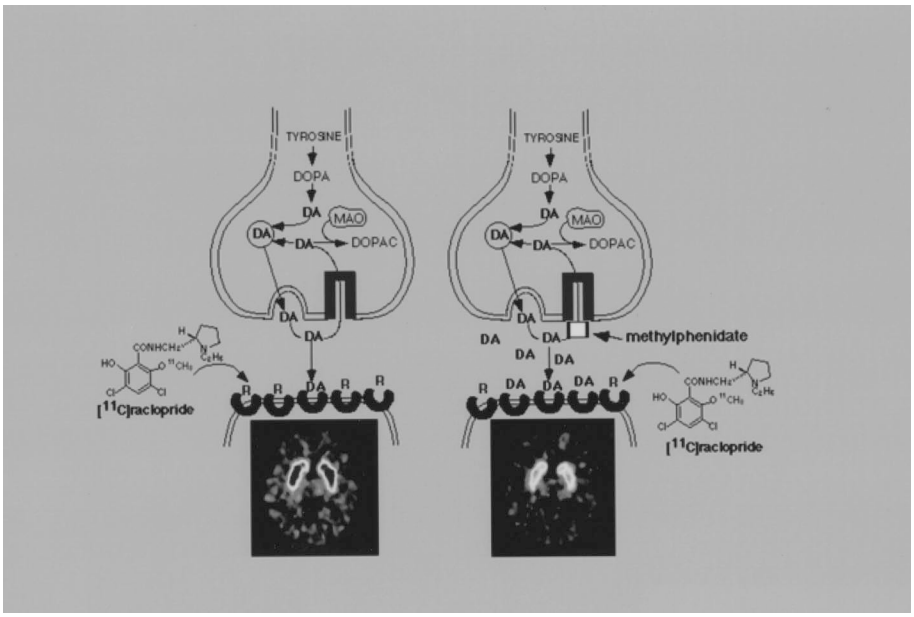

FIGURE 9 Use of PET to measure relative changes in DA concentration induced by methylphenidate with [^{11}C]raclopride. Methylphenidate increases synaptic concentration of dopamine and occupies DA D2 receptors; this results in reduction in [^{11}C]raclopride binding.

min apart to assess the effects of increases in DA concentration on regional brain metabolism. Parallel measurements were obtained of DA D2 receptors with [^{11}C]raclopride to evaluate the extent to which the changes in regional brain glucose metabolism induced by methylphenidate could be related to the concentration of DA D2 receptors in a given subject. It was shown that methylphenidate's effects on global brain metabolism varied among subjects, increasing it in 6, decreasing it in 2, and having no effect in 7 subjects but consistently increasing cerebellar metabolism in all subjects. As a result, none of the regional changes was significant (except in the cerebellum). The metabolic changes induced by methylphenidate in the cerebellum and in the frontal and temporal cortices were significantly correlated with D2 availability. Frontal metabolism and temporal metabolism were increased in subjects with high D2 receptor density and decreased in subjects with fewer D2 receptors. The significant association between D2 receptors and metabolic changes in frontal and temporal cortices and in the cerebellum suggests that methylphenidate's metabolic effects in these brain regions are due in part to DA changes and that the difference in D2 receptors is one mechanism accounting for the variability in response to methylphenidate. The results also show that changes in brain DA concentration, as induced by methylphenidate, can lead either to increases or to decreases in cortical and subcortical metabolism. That corresponds well to the recognized role of DA as a neurotransmitter that modulates the activity of brain regions, enabling both excitatory and inhibitory signals.[49] The significant correlation between methylphenidate-induced changes in metabolism and D2 receptors suggests that the effects of DA on brain activity depend in part on the state of the DA system, as had been shown in laboratory animals.[50,51] The dependence of the response to methylphenidate on the state of the DA system could help to explain differences in vulnerability to psychostimulant administration.

Metabolic studies have also been done to assess the effects of chronic administration of drugs—cocaine, alcohol, and marijuana—on the human brain. The studies have shown different regional patterns of brain metabolic abnormalities, depending on the substance abused, and have shown different levels of recovery from the metabolic abnormalities on detoxification. For example, chronic cocaine administration and alcohol administration are associated with a marked reduction in frontal metabolic activity. In cocaine addicts, hypofrontality

persisted even after protracted withdrawal;[52] in alcoholics, there was substantial recovery or detoxification.[53] For cocaine abusers, the reduction in frontal metabolism has been shown to be accounted for in part by the decrease in DA brain function.[53]

Cerebral Blood Flow

Studies with CBF have been particularly useful in documenting cerebrovascular toxicity associated with substance abuse, as was initially documented with PET in by studies that showed widespread decrements in CBF in cocaine abusers.[54] The imaging studies corroborated the clinical reports of cerebral strokes and hemorrhages in cocaine abusers and highlighted this toxic action of cocaine.[55] Because repeated studies can be done on the same subjects at different times, they can be used to evaluate the response to therapeutic intervention. For example, imaging studies have documented that the decrements in CBF in cocaine abusers improve with the administration of buprenorphine.[56] This exemplifies the value of imaging as a tool for assessing the efficacy of potential treatments for drug addiction.

CONCLUSION

Studies using PET have started to document the mechanisms of reinforcement of addictive substances and to delineate neurochemical changes in the brains of addicted subjects. The findings are still preliminary, but they indicate the potential of PET in substance-abuse research, including

- The direct assessment of the behavior of drugs of abuse in the human brain. This is relevant because pharmacokinetics and pharmacodynamics can vary among animal species. It also enables the assessment of drug behavior directly in drug-addicted subjects.
- The association of behavioral and neurochemical characteristics. Because PET studies are done in subjects while they are awake, they permit determination of the relationship between behavior and alterations in local neurotransmitter concentrations, metabolism, and flow. Studies can also be done to assess the relationship between the pharmacokinetics of a given drug and the time course of its pharmacologic effects.
- The comparison of biochemical characteristics and drug responses between addicted and nonaddicted subjects.
- Application in the development of therapeutic interventions for drug addiction.

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Discussant

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Metabolic Characterization of Oncologic Disease

Positron emission tomography (PET) uses a specific device, the tomograph, for imaging the distribution of a positron-emitting radiopharmaceutical substance that has been administered to a patient. The substance includes a radionuclide that is produced by a cyclotron or generator and incorporated into a substrate, which results in its localization. The substance most widely used for this purpose is fluorine-18 fluorodeoxyglucose (FDG). The 110-min half-life of ^{18}F permits its distribution from the site of production. A single regional site can supply FDG to multiple PET centers. PET has been demonstrated to have important applications in evaluating cardiac and neurologic diseases, but its major clinical application is in oncology, and its use in characterizing malignant diseases and the effect of therapy on them is increasing. In clinical oncology, FDG is used with PET to characterize glucose metabolism of tumors.

Instrumentation for PET has improved dramatically since its development by Michael Phelps and colleagues at the Mallinckrodt Institute of Radiology at Washington University School of Medicine in the early 1970s. Commercially available instruments have an intrinsic spatial resolution of 5 mm and simultaneously produce 30–45 contiguous slices with an axial span of about 15 cm. Imaging of an entire organ, such as the brain or heart, is now feasible from 1 bed position. Sequential longitudinal stepping of the data-acquisition software has made it possible to obtain PET scans of the whole body, which are particularly useful for oncologic applications. Recent developments in technology have resulted in dual-headed gamma cameras that can operate in a coincidence-detection mode that permits PET imaging. These instruments can also be used for routine nuclear-medicine studies. As this new technology is developed further, the number of institutions that can offer PET imaging will increase dramatically.

ONCOLOGIC APPLICATION

FDG imaging of tumors is performed when the patient is fasting to minimize competitive inhibition of FDG uptake by serum glucose. The FDG emission scans are made about 45 min after the intravenous administration of FDG. A whole-body scan can be acquired in 30–45 min.

Intravenously administered FDG circulates in the blood and is transported across capillary membranes by a carrier-mediated transport process. FDG and glucose are phosphorylated by hexokinase, but FDG-6-phosphate cannot be metabolized through the glycolytic cycle and is accumulated intracellularly. Glucose-6-phosphate undergoes further metabolism through the glycolytic and Krebs cycles to CO_2 and water. L. Sokoloff and colleagues[1] have developed a mathematical model for relating the net transport of ^{14}C deoxyglucose (DG) and accumulation of DG-6-phosphate in tissue to glucose transport and metabolism. This model, developed for rats and autoradiography, has been extended to humans and FDG PET.

Visual interpretation of the images can be complemented by semiquantitative analysis in clinical studies with FDG PET. These techniques provide an index of glucose metabolism because the amount of ^{18}F in tissue at any time is monotonically related to glucose use. The standardized uptake ratio is a semiquantitative index that is obtained by normalizing the accumulation of FDG in the region of interest to the injected dose and patient body weight. This index of glucose metabolism can also be corrected for lean body weight (instead of total body weight) and serum glucose content, but these corrections have not been found necessary for evaluating some tumors, such as lung cancer.[2]

The first clinical applications of FDG PET were in the brain. G. DiChiro and colleagues[3] demonstrated that the amount of accumulation of FDG in brain tumors distinguished histologically aggressive and less-aggressive tumors and could distinguish viable tumor and necrotic tissue after treatment. Many other tumors have been

studied with FDG PET. Although the largest number of clinical studies with FDG PET have been reported in lung cancer, a large body of information is available on several other malignancies. FDG PET has been demonstrated to be accurate for evaluating head and neck cancer, colorectal cancer, melanoma, lymphoma, breast cancer, hepatic neoplasms, and pancreatic cancer.

APPLICATION OF FDG PET IN LUNG CANCER

Background

The frequency of lung cancer is increasing worldwide. It is the most-common fatal cancer in men and women in the United States, where it claims 145,000 lives each year. The diagnosis and treatment of patients with lung cancer are fraught with problems that benefit from the use of PET. PET is particularly useful in a patient who has a solitary pulmonary nodule (for determining whether it is benign or malignant), in a patient who has documented lung cancer (for determining whether it has metastasized), and in a patient who has had lung cancer and therapy and has a residual mass (for determining whether the mass is cancer or a posttherapy scar).

Clinical Results

FDG PET has been demonstrated to be very accurate for evaluating pulmonary nodules. Most pulmonary nodules are detected with chest radiography, and patients with such nodules generally are referred for computed tomography (CT) for further evaluation. The nodules are generally indeterminate for malignancy on the CT scan. Some characteristics might lead to a diagnosis of a benign or malignant nodule, but most nodules are indeterminate. In an initial study from our institution, we demonstrated 100% sensitivity and 89% specificity of FDG PET for evaluating indeterminate nodules.[4] A recently performed multi-institutional study has validated the high sensitivity and specificity of FDG PET for characterizing indeterminate nodules. Before the use of FDG PET, patients with these nodules went to biopsy or thoracotomy for a definitive diagnosis. In surgical series, 30–50% of the nodules that were removed at surgery were benign. The cost effectiveness of PET for evaluating solitary nodules has demonstrated a large savings nationally because its use precludes unnecessary surgery.

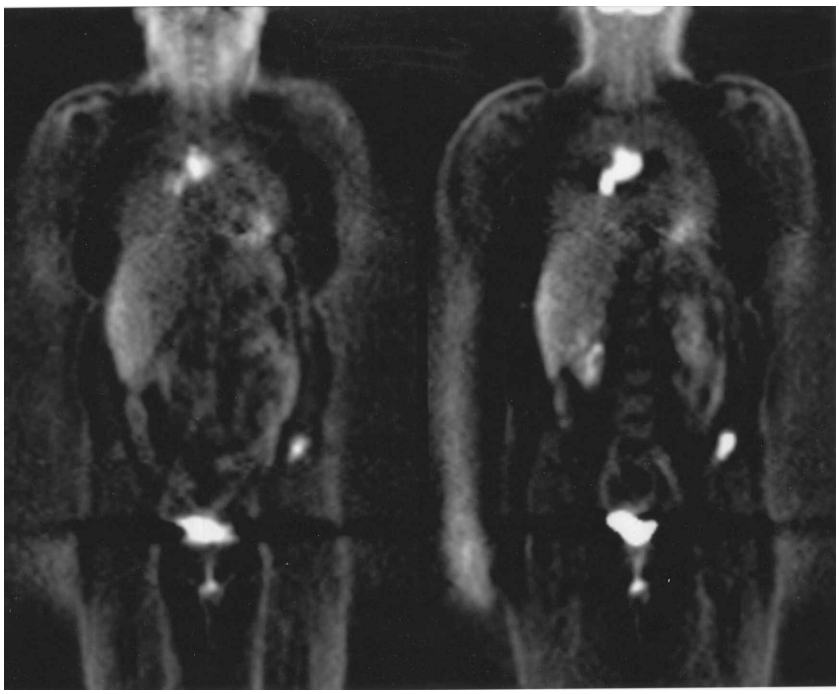
Lung cancer spreads first to the lymph nodes in the hilar areas and mediastinum. Autopsy studies have demonstrated a relationship between the size of lymph nodes and the likelihood of malignancy. However, small lymph nodes can contain cancer, and not all large lymph nodes are cancerous. A radiology–diagnostic-oncology group study compared CT and magnetic resonance imaging (MRI) for staging the mediastinum. These procedures were found to have accuracies in the 60–70% range. Several studies have demonstrated an 80–90% accuracy of FDG PET for staging the mediastinum, and these studies have demonstrated that PET is more accurate than is CT or MRI for staging the mediastinum.[5–7]

Whole-body imaging with FDG PET provides information that cannot be obtained with any other imaging technique. It has been shown to be very effective in detecting distant disease that is not identified otherwise and in clarifying whether a tumor is present when other techniques yield abnormal results. We have recently shown that FDG PET is very accurate in identifying metastases to the adrenal glands, which are common sites for the spread of lung cancer.[8] Other studies have shown that management changes occur in 30–40% of patients who undergo whole-body scans.[6,9] The whole-body PET study is replacing multiple-imaging studies for staging malignancies (figure 1).

CT and MRI cannot adequately characterize posttreatment pulmonary abnormalities as persistent tumor, scarring, or necrosis. A tissue biopsy that is negative for tumor cannot be accepted as definitive, because of limitations in the accurate sampling of regions of tumor in the midst of scarring. Patients who have chest radiographic findings that indicate tumor recurrence can be accurately characterized with FDG PET[10]. Not only can PET be used to evaluate tumor survival, but it can also be used to evaluate distant recurrence through whole-body scanning.

A recent study has documented the cost effectiveness of whole-body PET in the evaluation of patients who have lung cancer.[11] The use of FDG PET with CT results in large savings in health-care expenditures because of the avoidance of unnecessary surgery. These savings are realized without any effect on life span.

(A)



(B)

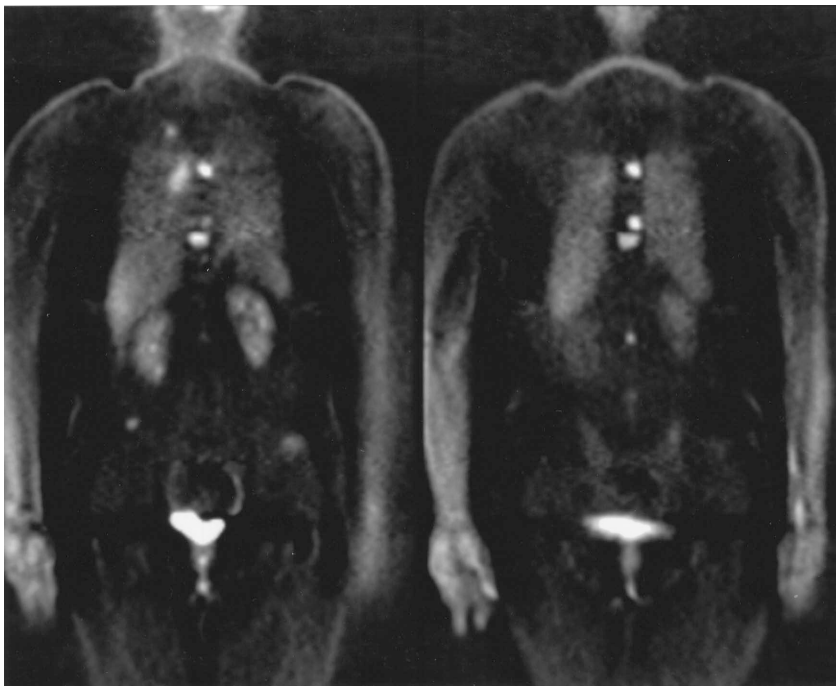


FIGURE 1. Selected coronal images from whole-body FDG PET scan of a 45-yr-old woman who presented with solitary pulmonary nodule and no other evidence of cancer or metastatic disease. (A) Images through anterior portion of body reveal metastases to lymph nodes in chest and to pelvis. (B) Images through posterior portion of body reveal small lung cancer in right lung and multiple vertebral body metastases.

SUMMARY

FDG PET is being used widely for characterizing neurologic and oncologic disease. Its use continues to increase as more data become available. Although initially used for characterizing the degree of malignancy of brain tumors and the effect of therapy on brain tumors, FDG PET is now being used to characterize most malignancies of the body and to follow the effects of therapy. Whole-body FDG PET is an excellent, cost-effective way to detect and stage malignancy.

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Discussant

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Michael Phelps has described the principles of positron emission tomography (PET) and demonstrated how this technique can be used to monitor biochemical processes in living subjects. Our goal is to marry this powerful imaging procedure with modern molecular and cellular techniques to develop new paradigms that can be used both in basic research and in clinical applications. I will summarize the essentials of PET analysis necessary for the understanding of the proposed research, describe some of the striking accomplishments in studying the consequences of altering the genomes of experimental animals, describe some of the methods currently used by molecular and cell biologists to image the expression of reporter genes, and describe the attempt to merge these molecular and cellular procedures by using reporter genes for noninvasive imaging of gene expression in living animals.

PET IN ANALYZING BIOCHEMICAL FUNCTIONS IN LIVING SUBJECTS

PET imaging uses positron-emitting molecules that are injected intravenously in trace quantities to allow us to observe and measure the biochemical functionality of tissues in living subjects. Typically, either positron-labeled ligands for cellular receptors or positron-labeled substrates for intracellular enzymes are used as PET probes. Retention of the positron-labeled PET probe in target tissues is detected with the tomograph, as a result either of the radiolabeled ligand binding to a receptor or of the conversion of the radiolabeled enzyme substrate to a "trapped" metabolic product. In the case of positron-labeled ligands for receptors—such as 3-(2'-[^{18}F] fluoroethyl) spiperone ([^{18}F]FESP), a ligand for the dopamine (DA) D2 receptor—the ligand is injected intravenously, and images are collected dynamically in the tomograph. Tissues rich in D2 receptor bind and retain the [^{18}F]FESP ligand and are identified in living subjects with tomographic analysis. In the case of positron-labeled substrates for enzymes, the injected substrate is converted to a product that cannot escape from cells. For example, [^{18}F]FDG is transported into, phosphorylated in, and retained in tissues in proportion to the glycolytic rate.

ADVANCES IN UNDERSTANDING OF GENE EXPRESSION IN VIVO AND OF CONSEQUENCES OF ALTERING PATTERNS OF GENE EXPRESSION IN LIVING ANIMALS

Role of Transgenic Mice in Study of Normal And Abnormal Phenotypes

It is now possible to add genes to mouse embryos to create mice that can express human genes. One of the first and most-dramatic examples is a mouse that overexpresses the growth-hormone gene.[1] Overexpression of a human growth-hormone gene in the germ line of the mouse caused mice to grow to sizes substantially greater than those of littermates that did not have the transgene.

If we place genes that are altered in human diseases into mouse embryos, we can often make murine models of human diseases. For example, many patients suffer from leukemia as the result of a rearrangement of the genes that encode for antibodies. When a rearranged "oncogene" is placed in the germ line of mice, the animals develop leukemia.[2] Such experiments satisfy a modern version of Koch's postulates, in that the rearranged gene is a causal agent in the induction of leukemia.

Use of Mice Carrying Human Genes Responsible for Diseases to Test Potential Therapies Without Ethical and Legal Problems Encountered in Studies of Human Patients

The gene for amyotrophic lateral sclerosis (Lou Gehrig disease) causes a similar degenerative disease of the peripheral nervous system in mice.[3] Studies on these mice have accelerated the regulatory approval of several drugs to modulate the effects of the human disease.[4]

The altered portion of the human gene responsible for Huntington disease has recently been transferred into mouse embryos. The animals carrying the Huntington mutation develop tremors, weight loss, and so on, and appear to develop a disease similar to that observed in humans.[5] Plans are under way to begin tests of a variety of therapies for the mouse disease.

Homologous Recombination to Substitute Altered Sequences for Endogenous Genes of Mouse Germ Line: An Important New Method to Analyze Gene Function in Living Animals

We now have the ability to change individual nucleotides or base pairs of a given gene in a mouse embryo (as opposed to inserting a human gene) to create mice that have the chosen substitutions—mice that will pass on a synthetic mutation to their progeny. We can directly alter the 1 or 2 nucleotides of our choice in the 3 billion base pairs that make up the mouse genome. To illustrate the nature of this accomplishment, if each nucleotide were 0.1 in. long, the line that represents the mouse genome would wrap twice around the earth—50,000 mi. By using our ability to make such specific substitutions to create mice with these changes in their genes, we can model many human diseases. For example, we can create mice that have the gene for cystic fibrosis. Those mice can be used to study responses to therapy.[6]

Sometimes we get surprised by the results of experiments. Michael Greenberg and his associates were studying the role of a gene called *FosB*, and they wanted to know whether this gene is essential for viability. When they made a mouse strain with a mutation in the *FosB* gene,[7] they found the animals to be normal in all respects that they assayed, except one: The mother mice would not nurture their young, and the babies perished unless transferred to foster mothers. That has led to the beginnings of molecular analysis of an entire behavioral process—nurturing behavior in mothers.

Those are just a few examples of how current cell biology and molecular biology have been extended into of the study of whole-animal physiology. How can these techniques be coupled with PET to allow the noninvasive study of the consequences of altering gene expression in living animals?

IMAGING TECHNIQUES CURRENTLY USED IN MOLECULAR AND CELL BIOLOGY TO STUDY REPORTER-GENE EXPRESSION

The light microscope revolutionized biology. Investigators could now see what had previously been only intellectual constructs. The electron microscope revealed entirely new dimensions of cell structure. Guided by the electron microscope, the field of cell biology emerged from biochemistry. The development of dynamic, noninvasive imaging techniques, such as PET, led to the marriage of imaging and biochemistry and allowed researchers and clinicians to study biochemical reactions in living animals and in patients.

Current Methods for Imaging Genome Function in Vertebrates

Developmental and behavioral biologists interested in imaging the expression of individual endogenous genes are restricted to immunochemical detection of protein products or in situ hybridization of mRNA. One must biopsy or, more commonly, sacrifice an animal in either case. Consequently, one usually obtains only a single "snapshot" from each subject.

Researchers studying the molecular basis of transcriptional gene activation use chimeric fusion-gene constructs in which the promoter–regulatory region of the gene under investigation is fused to a "reporter gene" whose protein product (such as luciferase or b-galactosidase) can be assayed biochemically or imaged with immu-

nochemical or histochemical techniques. The fusion genes are placed in the germ line of "transgenic" mice, rats, pigs, or sheep to create new strains of animals. Using site-directed mutagenesis, researchers alter the promoter–regulatory regions of fusion genes, create transgenic animals, and characterize the regulatory elements that control expression of individual genes in vivo. With these procedures, investigators can study the mechanisms of regulation of the individual genes that make up the genome. However, using current enzymatic, immunochemical, or histochemical analysis of reporter-gene products, one must sacrifice the animal; again, one obtains only a single snapshot from each animal.

Green Fluorescent Protein and Monitoring of Gene Expression in Living Preparations

All current methods to image expression either of endogenous genes or of transgenic reporter genes in rodents, primates, or humans qualitatively or quantitatively require the use of fixed tissue, either from biopsies or from sacrificed animals. Use of green fluorescent protein (GFP) as a reporter gene has recently provided a means to monitor expression of transplanted DNA in living cells repeatedly.[8] However, the utility of GFP as a reporter is limited to biologic preparations that are transparent to visible light, such as tissue-cultures cells, yeast, and *Caenorhabditis elegans*. Our goal is to develop imaging technology to monitor expression of reporter genes repeatedly in living experimental vertebrates and in patients.

DEVELOPING PET-BASED IMAGING TECHNIQUES TO EXAMINE REPORTER-GENE EXPRESSION IN LIVING SUBJECTS

PET Reporter-Gene–PET Reporter-Probe Systems for Imaging Reporter-Gene Expression in Living Animals

We are developing a means to "mark" transplanted cells or DNA delivery systems with PET reporter genes (PRGs)—genes whose protein products are either receptors for positron-labeled PET reporter probes (PRPs) or enzymes that metabolize PRPs. When animals or patients that express a PRG are given an injection of the corresponding PRP, cells that express the gene will sequester the probe. In contrast, cells that do not express the reporter gene will not retain the reporter probe (figures 1 and 2). Tomographic imaging will demonstrate the PRG-

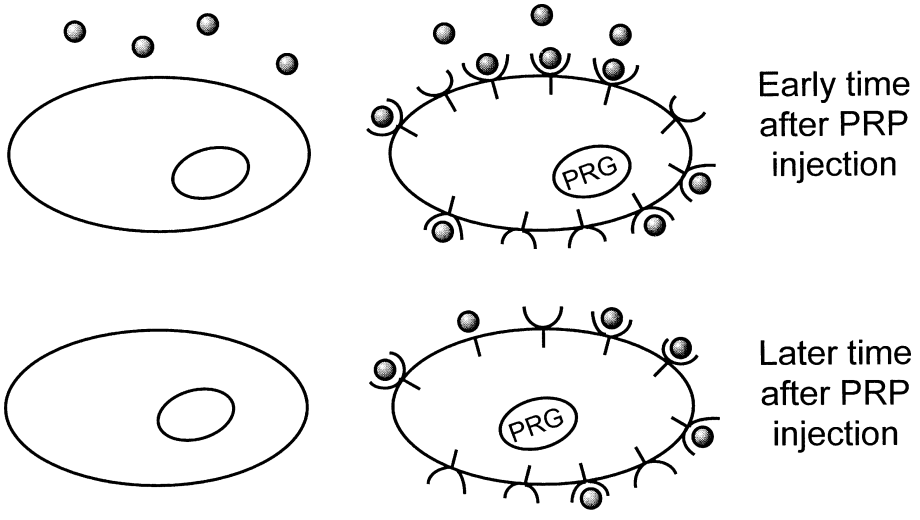

FIGURE 1 Sequestration of a positron-labeled PET reporter-ligand probe by a cell containing a PET reporter gene that encodes the ligand-binding receptor.

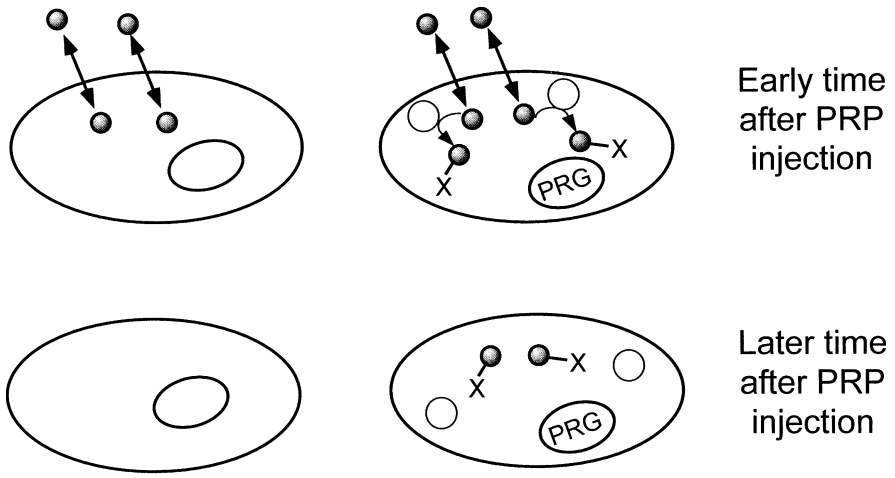

FIGURE 2 Sequestration of a positron-labeled PET reporter-substrate probe by a cell containing a PET reporter gene that encodes an enzyme that metabolizes the substrate.

dependent sequestration of the PRP. Experimenters and physicians will be able repeatedly to monitor, in living subjects, the tissue localization and level of expression of the PRG. Ideal PRGs should encode enzymes or receptors not normally present in mammalian tissues.

Herpes Simplex Virus Thymidine Kinase Enzyme and its Substrates for PRG–PRP Systems

The herpes simplex virus thymidine kinase enzyme (HSV tk) phosphorylates both thymidine and a number of guanine analogues (such as acyclovir and gancyclovir). In contrast, murine and human cellular thymidine kinases phosphorylate the guanine analogues only minimally. J.G. Tjuvajev and co-workers[9] have demonstrated the utility of HSV tk as a reporter gene in the mouse autoradiographically, using several radiolabeled guanosine analogues as substrates. We are working on procedures to use HSV tk and ^{18}F-labeled substrates as PRG–PRP combinations to image gene expression in vivo repeatedly and noninvasively.

DA D2 Receptor and FESP as a PRG-PRP System

We have used the DA D2 receptor as an alternative PRG first, because its normal expression at high concentrations is limited primarily to the brain; second, because a DA D2 receptor reporter gene will not be immunogenic; and third, because the nuclear medicine group the University of California, Los Angeles (UCLA), developed [^{18}F]FESP to image the DA D2 receptor in vivo in rodents, nonhuman primates, and humans.[10] The use of [^{18}F]FESP as a PRP requires no further research on probe development. Moreover, the mathematical tracer–kinetic model for this PRG–PRP system was developed at UCLA. We have developed the DA D2 receptor and [^{18}F]FESP as a PRG–PRP combination to image gene expression in vivo repeatedly.

Potential Role of Repeated Noninvasive Imaging of Gene Expression in Patient Care and Biomedical Research

The most-immediate potential practical application of the PRG–PRP procedure is in gene therapy. In 1 gene-therapy approach, cultured cells are transfected with a therapeutic gene and then transferred to the patient. In this adoptive-cell gene-therapy paradigm, it is difficult to determine whether the transplanted cells reach their target site, how long the transplanted cells remain viable, and whether expression of the therapeutic gene is later

compromised. In a second gene-therapy approach, DNA packaging and delivery systems (such as viruses and liposomes) that contain therapeutic genes are introduced directly into the patient; the packaging system is intended to target the therapeutic gene to the appropriate cells. Once again, after administration, the physician has no way to monitor localization or expression of the therapeutic gene. PRGs, administered in conjunction with therapeutic genes, will permit physicians to use PRPs and tomographic analysis for repeated monitoring of the localization, proliferation, and function both of cells used in adoptive-cell gene therapy and of DNA-delivery systems administered directly to patients. HSV tk, in conjunction with pharmacologic concentrations of gancyclovir, is being used in many active human-gene-therapy protocols. The availability of patients undergoing clinical trials for HSV-tk-based therapeutic protocols will facilitate investigation of the clinical utility of HSV tk as a PRG.

Reporter genes have been used in transgenic animals to analyze the relationship between genomic regulatory DNA sequences and gene expression and the effects of developmental and environmental manipulation on gene expression. In both contexts, analysis of reporter genes, such as b-galactosidase and luciferase, is limited to measurements at single times in biopsies or in tissue preparations from sacrificed animals. With PRGs and positron-labeled PRPs in transgenic animals, and new, high-resolution PET scanners dedicated to small-animal imaging, it will be possible to monitor repeatedly, in the same animal, time-dependent developmental, environmental, and experimental influences on gene expression.

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Health-Effects Research at the Crossroads: Molecular-Based Tools for Biologic Dosimetry and Individual Susceptibility

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These are unique and exciting times for health-effects research. Never before has such a wealth of readily exploitable materials, information, and technologies been available to bioscience researchers. The source of most of these resources has been the international human-genome effort. For the Department of Energy (DOE) Office of Biological and Enivronmental Research (OBER) program to remain competitive, it is essential that it reshape the direction of its health-effects research program. That can be achieved by capitalizing on the science and technology of the genome project. For example, a collection of 70–80% of all human genes is laid out before us now in the form of recombinant-DNA clones. With the unique technologies of molecular biology, bioinformatics, and bioinstrumentation, we can identify the genes responsible for susceptibility or resistance to radiation and chemical exposure and potentially use those genes as individual-specific biologic sentinels. In addition, those gene resources provide the basis for unraveling the mechanisms by which xenobiotic substances might cause ill health. We can also begin to consider large-scale studies to determine the structure and function of proteins, exploiting in the process the unique structural-biology resources that DOE has created. Technologies have arisen that enable miniaturization, massive parallel analysis, and exquisite sensitivity. Animal models are playing an increasing role in improving our understanding of the human gene function and its response to exposure. The current OBER health-effects program has a strong foundation in each of those fields and a proven ability to manage and conduct large-scale, complex projects effectively. The time is now to be bold, to capitalize on the strength of the current scientific program, and to develop and implement a vision for health-effects research in the 21st century.

EXPOSURE AND BIOLOGIC DOSE

Health-effects research began 50 yr ago in the Atomic Energy Commission, the original precursor of DOE, with a primary focus on measuring the health effects of ionizing radiation. Dose measurements were crucial then, and they still are. We know now that there are many steps between exposure and health effect. Exposure can come from the air we breathe, from the food or water we ingest, from our occupations, and from our lifestyles. Our exposures are moderated by factors within our bodies that convert external exposure to biologic doses. The factors that mediate this process can be the proteins that metabolize xenobiotic chemicals, proteins that repair damaged DNA, or other proteins that regulate our cellular processes and make us more or less susceptible to disease. Thus, over the last 50 yr we have learned that the dose measured by instruments is not necessarily the same as the dose that a person experiences. Moreover, 2 persons who receive the same dose might experience very different

biologic responses. We have learned that people are uniquely susceptible or resistant to heath effects. We have spent many years in developing sophisticated instruments that measure physical doses precisely, but their measurements might not reflect the biologic damage incurred by a specific person. That is the challenge to the health-effects program of the future: understanding the basis for individual susceptibility and using the information to minimize the health effects of exposure. The human-genome project will play a key role in identifying genes and providing technologies to address those issues.

We are very good at determining the shape of the dose–response curve at moderate to high doses, but we do poorly at low doses. We have not yet been able to define the part of the dose–response curve that covers the low doses. Why is that? It is not due to our inability to measure physical dose; our instruments are generally exquisitely sensitive. Rather, it is due to our inability to measure biologic doses with sufficient statistical certainty. We also generally observe a scatter in the points of the curve. Individual response—the unique ability of each person to detoxify, repair, or otherwise ameliorate a biologic insult—is probably a major contributor to that scatter. One of the venues for future research would be to understand the differences that make each of us more or less susceptible to effects of an exposure, whether to radiation or to chemicals. DOE can draw on its strengths in genomics and in cellular and structural biology and have a major role in reaching that goal.

Historically, DOE has made important contributions in developing tools for estimating biologic dose. The chromosome-painting technology was developed at a DOE national laboratory and has seen wide acceptance not only in basic research but also in clinical genetics.[1] The technology has become very useful for quantifying radiation damage in people and other living systems. It spun directly out of the genome project in that some of the first chromosome-specific painting probes were made from recombinant-DNA libraries established by the DOE-sponsored National Laboratory Gene Library Project in the 1980s.[2] Because the technology allows for relatively rapid analysis, many more cells or people can be examined for cytogenetic alterations than was practical with the previous conventional cytogenetic technology. That allows more statistical precision in measurement and facilitates quantification at lower doses. The technique has been applied to many population studies, including those of the atomic-bomb survivors[3] and the Chernobyl cleanup workers.[4]

If one desires to study the pharmacologic response or biologic consequence at the very low end of the dose–response curve, the accelerator mass spectrometry (AMS) methods developed under DOE sponsorship allow us to do it very well for chemical exposure.[5] The technology is used to count the number of carbon=14 atoms in the biologic material of interest. Cells in vito, in animals, and in humans can be provided ^{14}C-labeled chemicals at pharmacologically relevant doses to estimate the transport, detoxification, and conversion of the substance under investigation. Studies conducted with biologic AMS have been able to measure effects only one-hundredth or one-thousandth as large as those required by other laboratory methods. For example, it has been possible to measure the transport and fate of benzene in rodent systems at doses less than one-hundredth of the dose of benzene obtained from a single cigarette[6] or to measure the adducts formed in DNA from the eating of the equivalent of a well-done hamburger.[7] Being able to make measurements at such low doses has illuminated some major differences in pharmacologic response between high and low doses. Such methods as AMS offer a great advantage and might very well become the standard for chemical measurements in vivo as the technology matures.

INDIVIDUAL SUSCEPTIBILITY AND RESISTANCE

A person's genes play a major role in both response to exposure and susceptibility or resistance to disease. Genes encode the information to make proteins, and proteins perform various functions in cells. We need to place a much greater emphasis on understanding the structure of proteins, how they function, and how they might influence susceptibility or resistance to disease. Some of the relevant proteins are the cytochromes, which have a major role in chemical detoxification and modification in the liver and other tissues; free-radical scavengers that can protect us against radiation damage; and proteins that recognize and repair damaged DNA.

Examples of the role of proteins as susceptibility factors are numerous. A family of cytochrome P450 genes is clustered on human chromosome 19.[8] One of these genes, which encodes the protein coumarin hydroxylase, has been isolated and sequenced, and its variation in several population groups has been measured.[9]

The DNA sequence for this gene varies in the population. One variant sequence is caused by a single base change and results in loss of enzymatic activity; affected persons cannot metabolize coumarin or similar chemicals that use this pathway.

Another example involves DNA-repair proteins. ERCC2 is a gene involved in the repair of DNA damage. Defects in this gene are associated with 3 diseases.[10,11] The most severe is xeroderma pigmentosum D (XPD); those affected are extremely sensitive to sunlight and, in fact, develop cancers from such exposure. The second is Cockayne syndrome; the primary phenotype is severe neurologic dysfunction. The third is trichothiodystrophy which exhibits a milder phenotoype and is associated with metabolic deficiencies. The mutations that cause those 3 diseases all occur in ERCC2 and are complex and not well understood. There does not appear to be a single mutation associated with each one. It is known that the product of ERCC2 acts in a complex with other proteins. Therefore, it will be important to understand how proteins interact with each other and how mutations might affect interactions. A critical defect in 1 region of a protein might have a very different effect phenotypically from a critical defect in another region, depending on whether the regions are sites of interaction with other proteins.

In health-effects studies in the future, it will be important to make use of the resources and technologies that have already been developed to examine populations for genetic variation and to relate that variation to susceptibility and resistance to disease. The first step is to identify and sequence the appropriate suite of genes that might confer susceptibility. It is relatively straightforward to identify the 200–300 such genes that we know of today. Others will be added to the list as gene discovery accelerates in the human-genome project. The DNA sequence of the introns, exons, and 5´- and 3´-flanking regions for each gene must be determined. Although it is primarily the coding and regulatory regions that will influence protein production and function, a sufficient amount of intronic sequence is needed to facilitate diagnostics. The second step is to identify populations in which DNA-sequence variation in the genes can be measured. It is important to measure sequence variation not only in disease-susceptible and disease-resistant people, but also in the "normal" population. Subtle gene defects in many people might have more consequence for ill health in a population than would major gene defects in only a few people. Susceptibility genes can be resequenced with efficient, low-cost, state-of-the-art methods. Alternatively, given a sufficient number of regions to be resequenced, mass-hybridization methods could play an important role.[12,13] In fact, the "chip based" methods probably will be the methods of choice, once enough genes and potential variants have been identified.

Measuring a sequence variation alone is not likely to be sufficient for determining its role in ill health. Ultimately, we will need information on the consequences of sequence variation for the functions of encoded proteins. That will require a commitment to structural biology to determine the structures of the variant proteins and a commitment to biochemistry to evaluate functions. By making appropriate comparisons in normal, susceptible, resistant, and diseased people, we should be able to make the link between variation and disease. Appropriate animal or other model systems will be useful in validating the findings.

THE IMAGE CONSORTIUM

An important source of genes for studies on susceptibility is the IMAGE (intergrated molecular analysis of genomes and their expansion) Consortium.[14] This collaboration of industry, academe, and national laboratories has established the largest public collection of cDNAs and made them available worldwide to the scientific community. In addition to the clone resource, the ends of the cDNAs have been sequenced to provide ESTs (expressed sequence tags), and these are available in a public database, dbEST.[15] Over 600,000 cDNAs have been collected, and they are estimated to represent about 80% of all human genes.[16] Because these libraries represent material from over 30 human tissues and in many cases are highly redundant for specific genes or gene segments, one can begin to put together entire genes directly on the computer. Given that resource, it is possible to start with sequences known in other species to be involved in DNA repair or chemical detoxification and use them to see whether there are corresponding human genes. This will facilitate the identification and understanding of susceptibility and of other genes in humans. The cDNA collection also constitutes a powerful resource for studies on gene expression in normal and diseased tissue.

MOUSE AND OTHER MODEL SYSTEMS

Despite the tens of million of years of divergence between mouse and human, sequence homology is highly conserved. Comparative sequence analysis is a method for elucidating the functions of human genomic sequences by identifying sequence elements that are evolutionarily conserved between mouse and human. Sequence is expected to be more highly conserved for regions that represent essential biochemical functions. In addition to the coding regions, however, comparative sequence analysis can also identify other functional components of the genome, such as enhancers and other transcriptional regulatory elements. This approach is largely complementary to the sequencing of full-length human cDNAs, inasmuch as it is more likely to identify small, conserved noncoding elements, which are often well upstream of a gene or within introns and whose locations are nearly impossible to predict with conventional computational approaches. For example, the genomic region that contains the DNA-repair gene XRCC1 has been sequenced in both mouse and human.[17] The mouse and human genes each contain 17 exons, are 84% identical within the coding regions, and are 86% identical at the amino-acid-sequence level. Intron and exon lengths are highly conserved. In addition to the coding regions, 9 conserved elements were identified between mouse and human, with sequence identities ranging from 65% to 78%. It is interesting to speculate that these conserved noncoding regions are sites of regulation of XRCC1 and hence also play a role in its expression.

DNA sequence homology across species has been identified for genes associated with several human diseases. The human genes for cystic fibrosis, achondroplasia, and myotonic dystrophy share homology with sequences in the mouse, the fruit fly, worms, and yeasts. XPD, Huntington disease, and nonpolyposis colon cancer have corresponding mouse genes. Thus, other species can be useful models for studying the mechanisms of human diseases. For example, we can now begin to pursue a comparative genomics approach to targeted gene biology. Known sequences from other species can be used to query the cDNA database, identified human cDNAs can be obtained from the available IMAGE collection, and these cDNAs can be used as hybridization probes in human and mouse clones to identify the genomic region spanned by these genes. Simultaneously, the full-length cDNAs can be established and used for expression and functional studies. DOE has historically invested in such model studies, and this is a legacy of which it should be proud. With the information emanating at a high rate from the human-genome project, comparative mapping, sequencing, and functional analyses hold enormous promise.

BUILDING THE BRIDGES

The human-genome project has produced and will continue to produce excellent and extensive resources and technologies relevant to health-effects research. These take the form of genomic clones, cDNAs, DNA sequences, bioinformatics tools, automation, and instrumentation. We need to capitalize on those resources in the DOE health-effects research program. A major emphasis might be placed on using the resources to address individual susceptibility and health effects as an overarching theme. That would lead naturally to programs in gene action and function as related to the DOE mission, computational and structural biology for which DOE has extensive resources, and health and environmental studies. Now is the time to capitalize on the genome resources, and DOE can play a major role by applying its successful genomics experience to understand, prevent, and ameliorate health effects of environmental exposures.

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Discussant

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Major advances in our understanding of radiation effects[1] in the United States occurred in the latter part of the 20th century. Continued studies of atomic-bomb survivors, patients, workers, and the general public have provided new knowledge. Laboratory approaches are being incorporated into epidemiologic investigations to explain more about the biologic mechanism by which radiation causes cancer in humans. Despite great strides in understanding and quantifying radiation effects, there remains a lingering bemusement. Research that will continue into the next millennium might help to dispel perplexities as we build on and extend past accomplishments. The Department of Energy (DOE) and its predecessors and the National Research Council have contributed substantially to our knowledge of radiation effects.

RADIATION EPIDEMIOLOGY

Accomplishments in radiation epidemiology have been compiled and synthesized in the 1994 report of the United Nations Scientific Committee on the Effects of Atomic Radiation[2] and in the proceedings of the 1996 annual meeting of the National Council on Radiation Protection and Measurements (NCRP).[3] The focus of early studies has been extended and expanded. Leukemia was first studied in pioneering radiologists[4] and is now studied in nuclear-industry workers;[5] osteosarcomas in radium-dial painters[6] and now in retinoblastoma patients;[7] cancers after radiotherapy for ankylosing spondylitis[8] and cervical cancer[9] and now after radiotherapy for childhood cancer;[10] childhood cancers after prenatal X-rays[11] and now adult cancers after *in utero* irradiation;[12,13] thyroid cancer after thymic irradiation[14] and now after Chernobyl releases;[15] breast cancer after tuberculosis fluoroscopy[16] and now after Hodgkin's disease radiotherapy;[17] lung cancer among underground miners[18] and now among above-ground residents;[19] and cancer among atomic-bomb survivors[20]—and still among atomic-bomb survivors.[21] DOE and the national laboratories have supported key studies over the years, such as those of radium-dial painters, radiation workers, Chernobyl and other Russian workers, and atomic-bomb survivors. DOE has also been in the forefront of biodosimetry research, including classical cytogenetics, FISH technology, and GPA.

Occupational Studies

In 1902, the first cancer death attributed to radiation occurred after occupational exposure of Thomas Edison's assistant, Clarence Dally.[22] Except during radiation accidents,[23] such heavy exposures are rarely experienced today.

Studies of pioneering radiologists, underground miners, and radium-dial painters contributed to our understanding of radiation effects and to the setting of radiation protection standards.[24] Studies of radium-dial painters had been conducted for many years out of the Argonne National Laboratory. Radiation exposures in the workforce today are generally much lower than in the past, and any associated increases in cancer are difficult to detect. Combining and extending studies of occupationally exposed groups, similar to what has been done for underground miners,[18] appears to be the approach for the 21st century.

Nuclear-Installation Workers

Studies within the nuclear industry might be able to validate the risk estimates obtained from high-dose and high-dose-rate investigations. For many years, DOE has supported analytic epidemiologic studies of the workforce involved with the Manhattan Project and with later aspects of weapons and energy development. Radiation doses of most workers are recorded, and cumulative exposures of some might reach levels where any adverse effects could be detected. Nearly 100,000 nuclear-industry workers in 3 countries were analyzed, and leukemia, but not

other cancers, was statistically significantly increased.[5] Overall, only about 9 of the nearly 4,000 cancer deaths could be attributed to radiation. Even combined worker studies have difficulty in providing risk estimates of useful precision, however, when the range of exposures is narrow. Studies of electric-utility workers at nuclear-power stations might provide additional information on radiation risks because of the relatively high cumulative exposures possible.[25,26] More work is required to account for other potential hazards in the workplace, such as asbestos, solvents, and paints.

Plutonium Workers

Little correlation between plutonium intake and cancer mortality has been found in studies of large populations of workers exposed to low levels of plutonium. Studies out of Los Alamos National Laboratory have been informative in this regard.[27,28] Apparently, high-dose plutonium and gamma-ray exposures have caused excess lung cancers among over 2,300 workers at the Mayak radiochemical and plutonium production plants in Russia.[29-31]

Chernobyl-Cleanup Workers

Few epidemiologic studies of the more than 600,000 workers at Chernobyl who cleaned up the environment and entombed the damaged reactor have been published. Biologic-dosimetry studies confirm that the average dose received was about 10cGy,[32,33] although doses as high as 35 cGy were permitted because of the need for emergency action. Thyroid screening of 2,400 Estonian cleanup workers with ultrasonography and needle biopsy of nodules revealed no radiation-related increase in thyroid disease.[34] No excess incidence of leukemia or other cancers was found among nearly 5,000 workers from Estonia, and only death due to suicide was statistically significantly increased.[35] Much larger studies will be required to provide information on the effects of protracted exposures,[36] although a recent study of 155,000 Russian cleanup workers also failed to reveal a relationship between leukemia and recorded dose.[37] Continuing collaborative research in the Ukraine and elsewhere has been supported by DOE and other agencies.

Radiologic Technologists

Radiologic technologists who likely received much-lower total doses than did radiologists[38] have not been reported to be at increased risk for leukemia[39] or breast cancer.[40] A survey of 145,000 radiologic technologists in the United Stated should provide new information on radiation risks well into the next millennium, accounting for genetic susceptibility[41] and other cancer risk factors.[42]

Airline Crews

Airline crews are exposed to higher levels of cosmic rays than are earth-bound workers, in addition to a variety of physical and chemical carcinogens. Commercial pilots and flights crews might receive annual doses on excess of 5 mSv. Small studies of female flight attendants and male civilian airline pilots have been conducted.[43-45] If high-altitude supersonic travel is expanded, there is a potential for increased exposure of workers and of the general population to low-level high-LET radiation.

Atomic-Bomb Survivor Studies

The atomic bombs that ended World War II increased the rate of cancer in surviving populations.[21,46] These critically important studies of survivors have been and continue to be supported by DOE and the government of Japan. Risk was somewhat higher among children than adults, among residents of Hiroshima than Nagasaki, and among females than males. The excess relative risk over time has remained constant for adults but is decreasing for survivors exposed as children. The incidences of some cancers—chronic lymphocytic leukemia; non-Hodgkin's

lymphoma; Hodgkin's disease; and cancers of the pancreas, rectum, cervix, testes, and prostate—were not statistically significantly increased.

The dose response for leukemia appears linear–quadratic, whereas a straight line adequately fits the data for all solid cancers combined. About 500 cancers (accounting for 7% of all cancer deaths and 1% of all deaths) were attributed to radiation from the atomic bombs. Recent application of tumor-registry data revealed excess cancers of the liver, thyroid, and skin.[46] Little additional information will be obtained on Japanese survivors who were older than about 40 in 1945, inasmuch as most have died. The risk of radiogenic leukemia also appears to have run its course 40 yr after exposure. However, it will be well into the next millennium before lifetime risks among those who were exposed when young are fully assessed.

STUDIES OF PATIENTS GIVEN RADIOTHERAPY

Classic studies of populations medically irradiated for benign diseases (such as ankylosing spondylitis[8]) and malignant diseases (such as cervical cancer[47]) began in the 1950s and 1960s. Today, major advances in cancer therapy are allowing patients to live for many years, and the late effects of curative treatments have become important.[48] Studies of long-term survivors of Hodgkin's disease,[17] childhood cancer,[10] breast cancer,[49] and other cancers will continue to provide quantitative information on radiation risks that have both clinical and public-health importance. Studies of patients receiving whole-body irradiation for bone marrow transplants will be increasingly important as this modern treatment continues to cure patients. The largest combined study of over 20,000 patients who underwent bone marrow transplantations found substantially increased rates of second cancers attributable to high-dose, whole-body radiotherapy.[50]

STUDIES OF DIAGNOSTIC RADIATION

Other than radon, medical radiation contributes the most to population exposure. The first report that fractionated, low-dose radiation could result in breast cancer occurred in the 1960s, and modification of risk by dose and age was reported in the 1970s.[51] Radiogenic breast-cancer risk decreases with age at exposure, and risk appears minimal for asymptomatic postmenopausal women undergoing screening with X-ray mammography.[52] Periodic medical radiation exposures have not been linked to lung cancer and have been linked only weakly to leukemia. Fractionation appears to have little effect on reducing the risk of radiogenic breast cancer in tuberculosis patients repeatedly exposed to fluoroscopy, but it appears to reduce dramatically the risk of radiogenic lung cancer with a dose-rate effectiveness factor closer to 10 than to 2.[53]

STUDIES OF IODINE-131

It has been known since the 1950s that X-rays in childhood result in high rates of thyroid cancer. Exposure of the thyroid gland in adult life results in little if any cancer risk, whether from external photons[54] or internal I-131.[55] Thyroid cancer has not been linked to diagnostic uses of I-131, but results of a recent study suggest an association between thyroid nodular disease and I-131 administration.[56] The reactor accident at Chernobyl was followed by remarkable excesses of thyroid cancers after childhood exposure, which have been attributed to radionuclides of iodine, including I-131.[15,57–59] Other shorter lived isotopes of iodine possibly contributed to thyroid-cancer risk, as they did after the weapons-testing accident in the Marshall Islands in 1954.[60,61] The studies of Marshall Islanders have been supported over the years by the Brookhaven National Laboratory. The precise nature of the thyroid-cancer risk among the residents near Chernobyl remains elusive and awaits dose–response evaluations and clarification of the role of screening, the contribution of specific radionuclides, the modifying effect of endemic goiter, and the apparent urban–rural and country differences in risk.[57,62] The high excess within 5 yr of exposure seems to have come a bit too early to be consistent with results of prior studies.[54] However, if the experience of X-irradiated populations holds, the excess of thyroid cancer should continue far into the 21st century. DOE, in cooperation with the National Cancer Institute and other agencies, is supporting comprehensive studies of thyroid cancer in Belarus and other countries of the former Soviet Union. No increase in leukemia has been attributed to Chernobyl radiation[63,64] or to medical I-131,[65] in all likelihood because of the associated low bone-marrow dose.

STUDIES OF RADON AND RADON PROGENY

In 1556, Agricola described the mysterious lung disease afflicting miners of the Black Forest regions of eastern Europe, mentioning that the occupation was so hazardous that some women lost as many as 7 husbands to the dreaded condition.[66] Inhaled radon and radon progeny were later indicted as the culprits. Today, underground-miner studies are prominent in assessing the risk posed by low-level general-population exposures to radon,[67] although international residential studies might soon provide validation and guidance.[68] Studies of underground miners convincingly demonstrate that radon is a potent carcinogen, able to cause lung cancer and to interact with cigarette-smoking in a way that increases risk.[18] Estimates based on underground-miner exposures suggest that perhaps 10–14% of all lung cancers in the general population are related to breathing of residential radon. The possibility that radiation causes characteristic mutational features in lung-tumor cells has been investigated in several laboratories. It would be useful to be able to identify environmental carcinogens, including radon, on the basis of the genetic "fingerprint" that they leave on cells that become cancerous.[69-71] Leukemias and other cancers did not occur in excess among underground miners.[72]

It has been difficult to detect a radon risk in residential case–control studies largely because the relative risk at 150 Bq m^{-3} is so low, only about 1.15.[73] A recent study in Finland was negative despite a good design and relatively high exposure levels.[74] However, even that investigation did not have the statistical power to reject the possibility that miner estimates are appropriate. The value of combining data is seen in a recent meta-analysis of 8 large case–control studies.[19] Although the reasons for heterogeneity in risk estimates could not be clearly resolved, the combined estimate of the relative risk at 150 Bq m^{-3}, 1.14, was statistically significant. The authors conclude that, on the basis of current understanding, there is no reason to reject the validity of miner estimates, and future combining of current and continuing studies[75] will be required to evaluate fully the risk posed by indoor radon.

Large radon surveys have been conducted in the United Kingdom, where the average level of radon in homes is 20 Bq m^{-3}, and the action level is 10 times the average (200 Bq m^{-3}).[76] A small number of homes exceed 2,000 Bq m^{-3}, and 10,000 Bq m^{-3} was recorded in Cornwall. Although the importance of very low levels will continue to be debated into the 21st century, there should be no debate about the health hazard associated with the very high radon levels that can be experienced in some living quarters.

ENVIRONMENTAL-EXPOSURE STUDIES

Studies of populations exposed to high levels of natural background radiation have largely been negative or noninformative.[2,24,77] In general, ecologic studies are the weakest form of epidemiologic research and have provided little insight into radiation risks.[2,3,19] Unique studies of populations exposed to high levels of radioactive waste in the southern Urals of Russia, however, are being conducted and should continue into the next century.[78] In 1957, a storage tank at the Chelyabinsk nuclear facility exploded (the Kyshtym accident) and released large amounts of radioactive waste into the Techa River. Before the accident, high-level radioactive wastes from the Mayak facility were dumped into the river.[79] Leukemia has been reported to be in excess among the 28,000 residents,[79] and doses as high as 400 cGy were reported. The studies have the potential to provide new information on the effects of ionizing radiation delivered over long periods. DOE is supporting a number of important initiatives in the southern Urals.

STUDIES OF SUSCEPTIBILITY AND INTERACTION

In the 1920s, osteosarcoma was first identified as a consequence of radium poisoning from excessive ingestion during watch-dial painting among young women.[80] Today, radiogenic bone cancer remains a rare but important consequence of high-dose radiation exposure. Research into the 21st century is likely to clarify whether genetic susceptibility can increase the risk of radiogenic cancer, as among children with retinoblastoma.[7,81,82] Interaction studies might be increasingly important as we go beyond determining and quantifying radiation risks.[83,84] Do radiotherapy and chemotherapy together increase the risk of leukemia[85,86] or bone cancer[81]? Do

radiotherapy and cigarette-smoking increase the risk of lung cancer?[87] Do indoor radon and cigarette-smoking increase the risk of lung cancer as they do in underground miners?[18] Do stomach irradiation and surgery potentiate the development of stomach cancer?[88] Do radiotherapy and immunosuppression interact to increase the risk of lymphomas or other malignancies?[50] Is it possible that low-dose radiation can increase the risk of breast cancer in genetically predisposed persons? The evidence that heterozygosity for ataxia-telangiectasia (AT) can increase the risk of radiogenic breast cancer is not convincing.[2] Recently, it has been questioned whether even AT heterozygosity itself is an important risk factor for breast cancer.[89,90]

CONCLUSION

There will be some clarification of radiation risks in the next century—notably those associated with exposures in childhood, in medicine, and in particular occupations—as well as of the effects of I-131, plutonium, and radon. There will be advances in biologic dosimetry. There will also be new opportunities to study cancer mechanisms. Does radiation interact with environmental carcinogens or underlying genetic predispositions to increase risk? Will the cancer risk after childhood exposure eventually return to normal? Can radiation, whether external or internal, leave molecular signatures that implicate it uniquely as an environmental cause of specific cancers? Can we learn about susceptibility states as we learn about radiation effects? The research focus in the 21st century will build on the foundation that has been laid during the last 100 yr of radiation experience. Continued support from DOE will remain critical, as it has been in the past, to capture the scientific knowledge from studies of atomic-bomb survivors (the childhood exposures), studies in Russia (chronic versus brief exposure effects), and studies of biomarkers of exposure (FISH, GPA, and tooth enamel).

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What I want to do in 10 min is try to emphasize a couple of basic points related to one of the themes we have heard about today: the various paradigm shifts that are occurring.

We want to talk a little about risk-based decision-making and about the need to replace fear with scientific information. Much of our decision-making with respect to environmental and occupational factors has been driven by public fear. The scientific community has contributed to the development of that fear, but at the same time we have the paradox that we as scientists have been trying to create the scientific information that can tip the scales and let science and information weigh in the decision-making process.

The radiation arena, for example, illustrates what I'm pointing out here. Radiation is probably the risk factor that we know the most about, but it still ranks at the top of the list of public fears. Why is that? In our zeal to obtain funding to understand radiation, have we contributed to the development of public fear? In doing so, perhaps we have pushed aside concern for other risk factors.

We need to adopt a systems orientation as we look at human health risks, and our range of concern must extend from sources to human health responses. One of the contributions of the radiation community has been such a systems orientation. The understanding of internal dose came from the radiation field.

For many years, our approach to managing risks was pragmatic: we were attempting to get linkages between responses and exposures. Now for perhaps the last 15 yr, in a more-structured risk paradigm, we have attempted to focus on hazard characterization, hazard identification, dose-response relationships, and exposure assessments to characterize risk.

We might have given too much emphasis to identifying hazards without putting them into the perspective of overall risk characterization. But much of what has been developed with regard to that characterization has its roots in the radiation community and in the work that has been supported by the Department of Energy (DOE) and its predecessor organizations.

We have heard about the dilemma of detecting low levels of excess risk and the need to extrapolate. Typically, we are talking in terms of occupational studies, in which we can have confidence in data that show relative risks over 2. But we are squeamish when we get down to relative risks of 1.5. Today, we are starting to probe relative risks of around 1.05 and develop public policy based on them. In fact, our public-policy concerns go down into relative-risk regions where we must deal with extrapolation.

We can now measure not only the compounds in our environment, but what might be called punitive preneoplastic or predisease entities in the human body. How do we interpret those? Scientists have a special responsibility to point out clearly what we know but also to point out where we are extrapolating.

The other extrapolation involves going from the cells in vivo to the complex, integrated human organism. As our ability to probe molecular and cellular events increases, we move into a paradigm shift where we can no longer assume that every change is an adverse effect.

What happens in the complex integrated organism is an interplay of thousands of events. That puts a special responsibility on us as we try to interpret the results of our studies of potentially toxic agents in the cell system or in the laboratory animal system as they might apply to humans, frequently at doses that are much lower than those we are using here.

Dr. Hood has appropriately noted the extent to which we are going to have to continue to try to marry the field of laboratory science with the more population-based science that is needed for risk-based decision-making. A superb retrospective analysis by Samet and colleagues used Philadelphia data from 1974 to 1988. Philadelphia had a population of about 1.5 million. About 50 people a day died. Our challenge is to understand what factors are at play in causing such deaths, and what factors we can influence so that people can have not only a longer life, but also more-productive and more-enjoyable life.

We obviously are concerned that air pollution is one of the factors. Just as the deaths were of varied causes, the air pollutants were varied. But there are trends. If we examine the data closely, we see that some of them are interrelated. A major challenge is to tease out associations between risk factors and mortality patterns.

If we are going to make sense out of all this, we need improvements in the information base across the board, including information on individuals and on what they are dying of. Therein lies the continued tension between our desire collectively to have more information and our desire individually to have privacy. That is related to the ethical issue, and I am pleased to see that in the DOE program and in the National Institutes of Health program ethical issues have been given an important role.

We need to keep in mind that despite our attention to single risk factors, the real concern is not the small individual relative risks, but all the risk factors. A big cofactor in risk for many diseases is cigarette-smoking. We have educated a whole cadre of scientists who started to focus on particular risk factors as graduate students, and they have made a career out of studying those risk factors. They find it easy to talk only about those risk factors and forget the relative association. Radon in homes is a potential calculable risk factor, but it is important to keep in mind that about 80% or so of that risk is also attributable to cigarette-smoking. We need to talk about both, and we need to talk about the others that go along with them.

We have moved into an era in which we are trying to use risk assessments, make better use of risk-based decision-making, and make risk-management decisions. We need to continue to try to identify the research that will improve risk assessments; and we have to continue to seek means to communicate the total risk process and risk characterization to all stakeholders.

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Pity today's environmental-health policy-makers. Not only do they set policy that is guaranteed to make enemies—of those who feel that it is needlessly costly and those who feel that it is needlessly weak—but also they must make decisions on the basis of too little information. And in addition to their having too little information, much of the information that they have is not directly relevant to the real-world situations in which it is applied.

Intelligent environmental-health policy is a necessity. We as a nation have made great strides in studying and treating end-stage disease. Increasingly, though, we are coming to understand the costs of focusing so strongly on end-stage disease, both in terms of economic costs and in terms of the costs of human suffering. It would be much better to prevent diseases in the first place, a possibility that can be realized by better understanding and control of the environmental components of disease. Diseases arise from the complex interaction of our environmental exposures, our individual susceptibility to the effects of exposures, and the age or time at which we experience the exposures. Each of those components must be better understood; armed with a stronger scientific foundation, environmental-health policy that best serves real communities could be developed.

At present there are a number of bottlenecks to developing the ideal world of rational decision-making that would engender confidence among the American people.[1] Four of them will be discussed here: The National Institute of Environmental Health Sciences (NIEHS) is committing funds and effort to defining the parameters of *individual susceptibility* to individual toxicants, to developing innovative *toxicity tests* that are faster and cheaper that the current models, to exploring how chemical toxicity changes when toxicants are present in *mixtures*, and to *assessing* actual environmental *exposures* as they occur in a sample of the American people. This more complete knowledge base will greatly improve the tools used in designing environmental-health policy.

INDIVIDUAL SUSCEPTIBILITY

There is an urgent need to determine the genetic basis of individual susceptibility to disease, particularly to the environmental components of disease. To that end, NIEHS recently announced plans for a new Environmental Genome Project. It will be a multiyear, multicenter endeavor that will sequence the 200 or more genes that we think predispose people to environmentally associated disease. It will also be a transagency endeavor, requiring collaboration with the National Institute for Human Genome Research, the National Cancer Institute, and others. The first phase of this study is expected to last 5 yr and to cost $60 million. More details on this project are given at the end of this paper.

Clearly, we are not all equal, or homogeneous, with respect to our susceptibility to the effects of environmental exposures. Not everyone who smokes develops lung cancer, and not everyone exposed to carcinogens or toxicants gets sick. It is time to undertake a national effort to identify polymorphisms that account for this interindividual variation, relate the variation to population groups, and incorporate the resulting knowledge into commonsense, science-based knowledge of genes that code for P450 enzymes and DNA-repair enzymes, genes that direct cell-cycle control, genes for the metabolism of nucleic-acid precursors, and genes for signal-transduction systems. (Others in this volume, such as Anthony V. Carrano, have mentioned the importance of several of these genes.)

IMPROVED TOXICITY TESTING

There is an urgent need to develop toxicity tests that are faster and less expensive. The NIEHS National Toxicology Program (NTP) has been advocating this need for several years and has brokered collaborations with the Chemical Industry Institute for Toxicology, the Environmental Protection Agency (EPA), the Food and Drug

Administration, and a number of industry groups. To date, we have identified 2 transgenic-animal models that can identify carcinogens in 6 mo, rather than in the standard 2 yr.[2,3] The need for fewer animals and histopathologic slides also reduces the evaluation time of these studies, which currently extends them to 5 yr and more. Studies using these new models can be done at about one-tenth the cost of the 2-yr rodent bioassay.

The models are still being evaluated, but the results are encouraging. One boon to risk assessment is that the new models appear to minimize the strain-specific effects found in conventional rodent models, which so often confound the risk-assessment decisions based on them. On the basis of these successes, we have increased confidence in, and enthusiasm for, our ability to develop models that generate toxicity data faster and less expensively. It is our further hope to improve on even the 6-mo timetable. In the future, models can be developed with recombinant-DNA methods, combinatorial chemistry, knowledge of mechanisms, and structure-based prediction that could provide a high-throughput technology to identify carcinogens and other toxicants in a matter of days.

MIXTURES

Although toxicity studies are conducted on single agents, we are seldom exposed to 1 agent at a time. We live in an environment composed of a complex and changing mixture of natural and synthetic chemicals. Consider the number of chemicals in the air we breathe, in the water we drink, and in the food we consume, and you see how poorly toxicity studies capture the complexity.

The important question is, How do these agents interact? Synergistically, antagonistically, additively? We already know of several examples of synergism of carcinogens, such as that shown by concurrent exposure to cigarette smoke and radon[4] and, in the case of liver cancer, to aflatoxin B1 and hepatitis B virus.[5,6]

Clearly, then, we have evidence of synergistic interactions, both among chemicals and between chemical and physical agents. That possibility raises concern in light of current awareness that chemicals in our environment can have estrogenlike activity. These environmental estrogens individually have very weak estrogenic activity compared with our bodies' own natural estrogens. What is unclear is their synergistic or multiplicative potential, which will remain bothersome until we complete studies that validate or refute the possibility. At NIEHS, there are numerous efforts to elucidate the potential health effects of environmental estrogens. Furthermore, we plan to release a request for applications that will solicit university-based scientists to propose new studies in complex-mixtures research.

EXPOSURE ASSESSMENT

We do not know what chemicals the American people are exposed to. Exposures are estimated on the basis of EPA toxic-release and production-inventory databases, but the estimates indicate only the potential for exposure, rather than actual exposures. Real-world exposure-assessment studies are needed to address this information gap.

We are all different in our uptake and metabolism of environmental toxicants. We need studies to determine actual biologic exposures; that is, how much of a substance that someone is exposed to is absorbed and distributed systemically. We need to define the biologically effective dose for individual toxicants. We will probably discover that the biologically effective dose is different for different people; this is another aspect of individual susceptibility.

Exposure-assessment studies can and should be done now. NIEHS NTP is developing a partnership with the Centers for Disease Control and Prevention to incorporate more exposure assessment into the NHANES-IV study. Results will help to answer the question, Of the 70,000–80,000 synthetic chemicals in the United States, how many should we be worried about? Is the number 40? 50? 100? 200?

Exposure-assessment data can be analyzed in different ways to determine whether there are important differences in toxicant uptake between population groups. Results can be analyzed by sex, socioeconomic status, or age. Age is an important way to separate and analyze data because children's exposures can vary dramatically from those of adults, both because of their smaller body mass and because of behavioral differences. Analyzing the data

by socioeconomic status could also help us to determine whether the poor, because of where they live and work, have exposure profiles different from those of the rest of the population.

ENVIRONMENTAL GENOME PROJECT

The Environmental Genome Project was officially announced during NIEHS testimony before Congress at appropriation hearings in April 1996. The announcement was received with much enthusiasm and interest and has been the subject of numerous articles in the press.

Since then, NIEHS management has met with Harold Varmus, director of the National Institutes of Health; Francis Collins, director of the National Institute of Human Genome Research; Richard Klausner, director of the National Cancer Institute; and other institute directors. Many have committed funds to the project. In October 1997, we convened a working group and a study group to design the project and develop appropriate safeguards.

Basically, the project will involve sequencing the allelic variants of genes known to control various processes. The technologies developed by the Human Genome Project will enable the Environmental Genome Project to proceed more rapidly than previously has been possible. The multicenter project is expected to involve many of the same centers as did the Human Genome Project. We expect to sequence environmental-susceptibility genes in about 1,000 persons from several population groups. The study group will help to define the numbers needed to ensure a statistically reliable sample.

It is possible that 20–50% of the population will have mutations or polymorphisms of some of the genes that predispose them to disease arising from unique environmental exposures. If that is true, the impact on public health will be enormous. The taxpayers' return on understanding frequently occurring genetic polymorphisms that lead to disease will obviously be much greater than that generated by sequencing and studying rare-disease genes that affect, at most, 1% of the population. Thus, the Environmental Genome Project has the potential to have a tremendous public-health effect.

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Discussant

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The core of structural biology in this country now lies in the unique facilities provided by the Department of Energy (DOE) for the scientific community. The premiere technique is X-ray diffraction, particularly the immense capabilities provided by the synchrotron radiation sources at Brookhaven, Berkeley, and Argonne national laboratories and at Cornell and Stanford universities. Synchrotron radiation sources have 2 major advantages over the conventional X-ray sources available in the laboratories of academic and industrial scientists: The finely collimated synchrotron X-ray beams are at least hundreds of times more intense than conventional X-ray sources, and the wavelengths of synchrotron X-rays are tunable. These capabilities have enormously extended our ability to solve the structures of proteins, nucleic acids, and their multisubunit complexes rapidly.

Several other techniques complement these X-ray capabilities, particularly the neutron beams provided at Argonne, Brookhaven, and Los Alamos national laboratories. X-ray diffraction can locate the heavier atoms (such as carbon, nitrogen, oxygen, and phosphorus) in biologic macromolecules. In contrast, the hydrogen atom is a very weak scatterer of X-rays and can be detected only in the very few crystal structures of the highest resolution. Neutron diffraction complements X-ray diffraction because of its ability to locate hydrogen easily and provide the orientation of water molecules and the direction of hydroxyl groups, data that are important for understanding the catalytic and other functions of proteins and nucleic acids. In elucidating the low-resolution solution structures of large multisubunit protein and protein–nucleic-acid complexes, neutron scatter is the technique of choice because the scattering length of hydrogen has the opposite sign to that of the other atoms found in biologic macromolecules and also to that of deuterium. Those differences can be exploited to determine the spatial relationships of proteins and nucleic acids in complex biologic assemblies. In addition to the above applications, inelastic neutron scatter can be used to study protein dynamics and the role of dynamics in protein functions.

Multidimensional nuclear magnetic resonance (NMR) spectroscopy is used widely in academic and industrial laboratories to solve the solution structures of low-molecular-weight proteins and nucleic acids. This technique is limited to protein molecular weights up to about 30,000 daltons because of the spectral line broadening that accompanies the slower tumbling of larger molecules. The size limit can be extended by the specific labeling of proteins with stable isotopes provided by DOE and National Institutes of Health resources. It can be extended also by increasing the field strengths of the superconducting magnets used with NMR. The Environmental Molecular Sciences Laboratory at Pacific Northwest Laboratory is working with Oxford Magnetics to extend the magnetic-field strength to give a frequency of 1 GHz for protons, which is probably the limit of the current superconducting metal-alloy wire. This very high field strength, with specific labeling with stable isotopes, will provide users with the most-advanced capabilities for multidimensional NMR.

Other important techniques include the use of the high-field scanning transmission electron microscope at Brookhaven National Laboratory and high-field mass spectrometers at Pacific Northwest Laboratory. The emerging atomic-probe microscopies have the potential to revolutionize structural biology at low and intermediate resolutions. Current interest in these techniques stems from their ability to image hydrated biologic macromolecules adsorbed on flat surfaces. In addition to the structural information obtained, ligands can be attached to the probe tips and used to examine samples. Under development is a range of atomic-probe tips with different chemical and physical characteristics designed to address specific questions concerning the function of macromolecules, organelles, and cells.

The DOE structural-biology facilities (figure 1) provide resources for both internal and external users in the United States. The responsibilities of the internal users are to extend the capabilities of the facilities and to apply

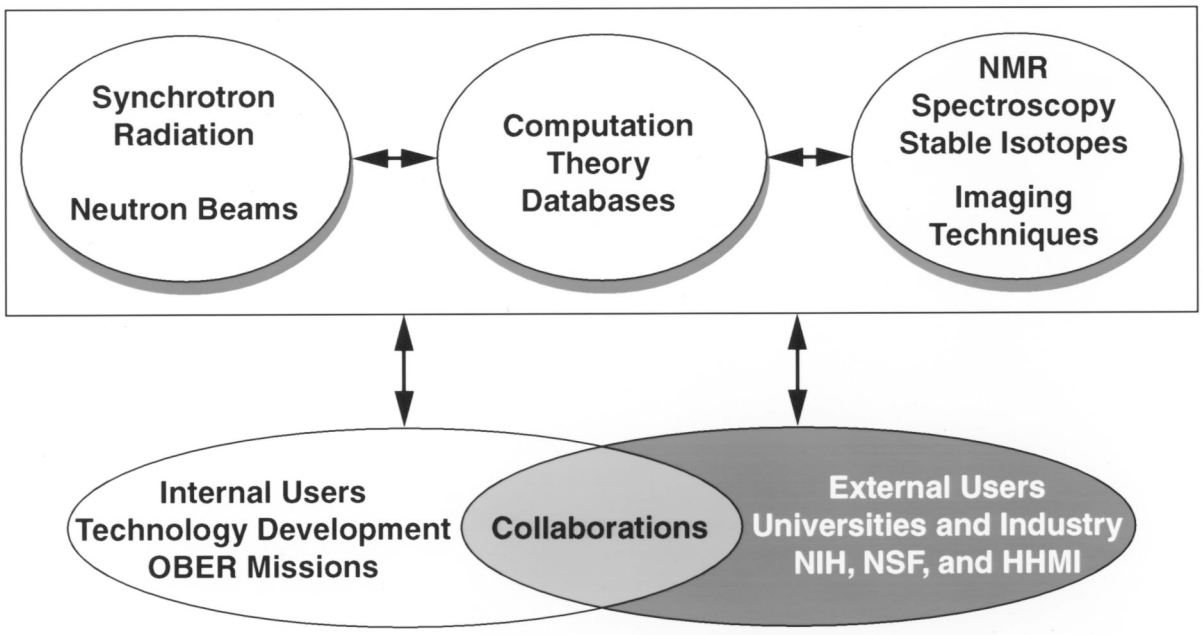

FIGURE 1 DOE structural-biology programs.

them to the research missions of the DOE. In addition, they provide an interface with external users to exploit the facilities efficiently and to collaborate on projects of joint interest.

Structural-biology capabilities similar to those available in this country are enjoyed by scientists in Europe and Japan. However, as discussed in an article in *Nature,*[1] there are serious concerns about the "neutron drought" in this country. The situation is exacerbated by the closing down of the High Flux Beam Reactor at Brookhaven National Laboratory; for the future of neutron science in this country, it is hoped that the shutdown does not become permanent. The neutron-spallation source at Los Alamos National Laboratory is being upgraded, but this will only partially alleviate the demand for neutrons by the scientific community. According to the *Nature* article, there are 3,000 regular neutron users in Europe, compared with 1,000 in this country. Most of the users have backgrounds in condensed-matter physics and chemistry. The smaller contingent of structural biologists uses neutron beams to extend the findings of X-ray crystallography by locating hydrogen atoms, water molecules, and ions and to determine the low-resolution organization of complex multisubunit protein and protein–nucleic-acid complexes.

In comparison with the neutron-beam situation, synchrotron radiation beams are becoming widely available. Synchrotron beams extend our capabilities beyond those of conventional X-ray sources in 3 ways: Regular crystal structures can be solved more rapidly; the structures of biologic macromolecules and their complexes that give small or irregular crystals can be solved; and the structures of complex biologic assemblies, such as viruses and multisubunit complexes, can be solved in a reasonable period. The third is very important because the major trend in biomedical sciences is toward increased complexity. All aspects of DNA processing—transcription, replication, and damage repair—involve multiprotein-DNA complexes. The process of transcription in yeast requires no fewer than 50 proteins. In addition, many of those proteins are subjected to reversible chemical modifications, such as phosphorylation. How do we approach such complexity? The starting point for the ultimate understanding of such complex processes includes the structures of the individual components and of their complexes at different stages. Similar structural and temporal complexities are involved in most biologic processes, and there is an urgent need to develop approaches to explain these complexities and the rules governing the assembly of complex biologic systems.

The initiation of the Human Genome Project by the Office of Health and Environmental Research, now the Biological and Environmental Research (BER) program, resulted from recognition that the full understanding of the health effects of DNA damage by radiation and chemicals would require detailed knowledge of the sequence organization and packaging of the human genome. The project has developed into major national and international genome programs, and the sequencing of the human genome is expected to be completed within the next decade. More recently, BER initiated a project directed at sequencing the genomes of microbes relevant to the missions of DOE in the environmental sciences, in environmental remediation, and in the development of responses to biologic threats. Progress in the human- and microbial-genome projects has far exceeded expectations, and sequence data are rapidly being accumulated. Cognizant of these successes, the scientific community is discussing the exploitation of the science embodied in the sequence data. With the increased capabilities in synchrotron radiation facilities, an explosion in the number of structures that will be solved by X-ray diffraction can be predicted with confidence; this has major relevance to the human- and microbial-genome projects and depends on their success.

A major structural problem associated with deciphering the human genome concerns the structure–function relationships of the proteins coded by the very large number of unknown genes in it. Most structures under investigation are of proteins with known functions. These make up a small subset of the proteins coded by the estimated 60,000–100,000 human genes. The problem of understanding the functions of such a large number of unknown proteins is formidable and will be the task of the functional-genomics component of the Human Genome Project and of molecular and cellular biology. The initial phase of functional genomics is to devise strategies to provide information on the possible functions of unknown proteins; for example, an antibody to an unknown protein would allow its subcellular location to be determined. A structural approach to functional genomics would involve the identification of the complete alphabet of structural motifs that are required for specific protein functions, such as DNA binding. From the identification of particular sequence–structure motifs, the possible functions of unknown proteins might be predicted from their gene sequences. In biology, however, there are always exceptions to general rules, and proteins with quite different structures have evolved with identical functions. As proposed by Joel Berendzen and Thomas Terwilliger of Los Alamos National Laboratory; David S. Eisenberg of the University of California, Los Angeles; and Sung-Hou Kim of the University of California, Santa Barbara, the long-term rational approach to functional genomics would be a major national and international effort to determine a representative set of protein structures that would provide the database to model or predict the structures of any protein on the basis of its gene sequence. A structure-determination project of this magnitude could be organized as a protein-structure initiative.

The purpose of such a protein-structure initiative would be to obtain the structures and functions of proteins from their sequences. Such an initiative would require a coordinated effort to obtain the required structural database and informatics. With such a coordinated effort, the "basis set" of protein structures required to predict unknown structures at a coarse level could be obtained in 5–10 yr. A more-comprehensive basis set, which would allow the precise prediction of these structures from their sequences, might take 10–20 yr. The impact of such databases of structure would be enormous for the biotechnology industry and the health industry and for our fundamental understanding of how enzymes and other biomolecules function. Moreover, the comprehensive basis set will provide structural and thereby functional information about genetic polymorphisms and how they contribute to individual susceptibilities to environmental stresses. Existing protein-structure databases should be coordinated with the Human Genome Project in determining priorities for protein-structure determinations. Determination of the structure of an unknown protein would have priority over determination of the structure of a new member of an existing protein family. A protein-structure initiative would require large-scale capabilities for the overexpression, purification, and crystallization of the many unknown proteins identified by the Human Genome Project.

As mentioned previously, a major direction in the biomedical sciences is toward increased complexity. In biologic processes that involve a large number of known proteins assembled into multisubunit protein complexes or protein–nucleic-acid complexes, pooling strategies, such as those proposed by David Torney (Los Alamos National Laboratory), are required to determine the order of assemblies of such complexes. In the case of a protein of unknown function, it is necessary to determine whether it functions alone or in combination with other protein

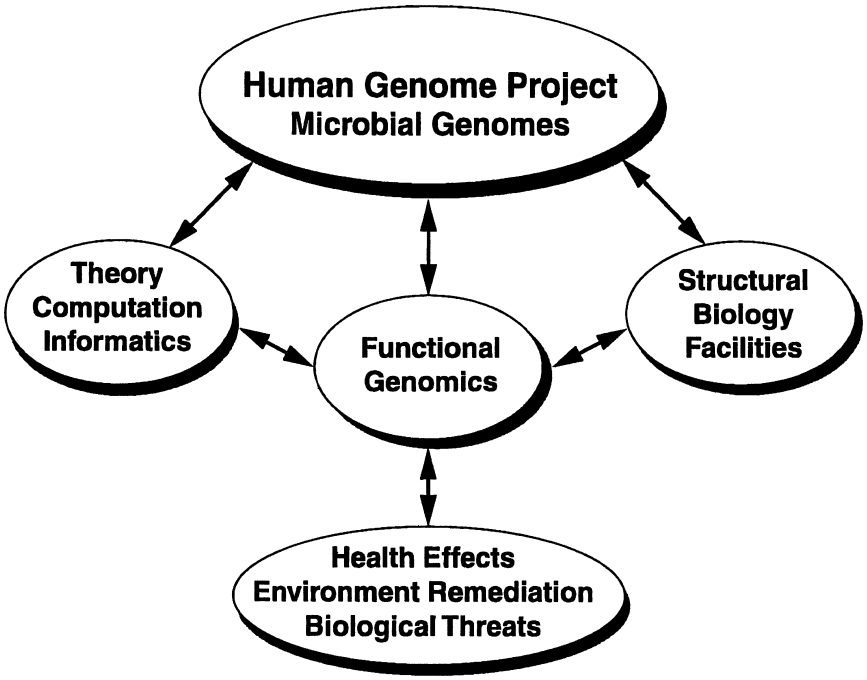

FIGURE 2 Relationships of DOE facilities and capabilities.

subunits. The identification of a conserved cluster of residues forming a patch on the surface of a protein would be a strong indication that such a patch is involved in interactions with other proteins that would have to be identified. Experimental approaches that can be used for this purpose include the yeast 2-hybrid system and phage-display techniques. Phage-display techniques provide powerful approaches for functional genomics. Phage random-peptide and gene-fragment libraries can be used to identify interacting peptides and proteins. Phage-antibody libraries provide an easy and very rapid approach to the derivation of antibodies against antigens, including those that are highly conserved and difficult to obtain for biologic reasons. This approach is ideally suited to functional genomics and the selection of antibodies against toxins.

In addition to the large number of unknown genes that will be found by the Human Genome Project, unusual noncoding DNA sequences will be identified. For example, the expansions of triplet DNA repeats define a new type of mutation, a DNA structure mutation, in the human genome. The genetic instability due to triplet-repeat expansions is associated with many inherited neurologic diseases. Expanded-triplet repeats are found inside noncoding DNA regions; upstream, downstream, or in the introns of genes; and, less often, in coding-DNA regions. Unusual DNA structures, such as DNA hairpins, can be formed by the DNA strands of repeated trinucleotide sequences upstream or downstream of genes and probably affect the expressions of associated genes. These mutations, therefore, affect not the intrinsic function of the gene product, but the level of its expression. Either the associated gene is totally suppressed or the expression of its protein product is reduced to nonphysiologic levels. When the repeat is a triplet codon and is in the coding region of a gene, it introduces a run of amino acids into the protein, thereby affecting its function. Many unusual noncoding-DNA sequences have been found and will continue to be found by the Human Genome Project, and it will be necessary to determine their structure–function relationships.

Finally, the Human Genome Project will identify DNA sequences involved in essential chromosomal elements, such as telomeres, centromeres, and origins of DNA replication. Because of the perceived links of the

shortening of telomeric DNA repeats with aging and the reactivation of telomerase (the enzyme that maintains the length of telomeric DNA) with rapidly dividing cancer cells, the functions of telomeres are under intense investigation. In addition to the above structural elements, DNA sequences required for the long-range organization of the human genome in chromosomes will be identified by the Human Genome Project. Those sequences are thought to define and isolate DNA loops or chromosomal domains as both structural and genetic units. It is important to note that there is no cross-talk between individual genetic units and the control of individual genes. This phenomenon is determined by the interactions of *trans*-acting factors with *cis*-acting DNA-sequence elements and by the topologic state of the DNA loop. It is probable that new sequence elements required for control of gene expression will be identified. Clearly, the structure–function relationships of chromosomal and genetic sequence elements will have to be elucidated for a full understanding of chromosomal organization, structure, and function.

There is little doubt that the future of structural biology in this country lies in the unique facilities provided by DOE. A broad structural-biology initiative would link the capabilities of these facilities with the human-genome and microbial-genome projects through functional genomics and health-effects reasearch as outlined in figure 2.

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Discussant

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INTRODUCTION: THE MODEL MOUSE

The mouse has a long history as an experimental model in genetic and toxicity studies because it is small, breeds rapidly, and survives well in the laboratory setting. Mice begin to reproduce at the age of about 6 wk, and a female mouse typically produces large litters—10–20 offspring—every 6 wk for the remainder of her reproductive life. A mouse's life span is relatively short, so it is possible to examine large numbers of animals of a particular lineage over the full course of their life cycles. Because both genetics and toxicology are statistical sciences that require data from many individuals for accurate results, mice have been the model of choice for these (and other) fields of study for many years.

Many sophisticated methods of genetic and embryologic manipulation have been developed in the mouse. The most-promising and important of these methods are transgenic techniques, such as gene targeting, which has been exploited very successfully in the last several years. Gene targeting has revolutionized the use of mouse models by permitting us to create mutations in genes that are known or thought to be involved in human disease. Once generated, the mutant mouse can be used to monitor the progress of a disease and to test its response to experimental treatments without subjecting human patients to risk or discomfort. Others have discussed the details of gene targeting and provided examples of how mouse targeted mutations can be used to study human diseases. My focus is on other types and uses of "model mice."

VARIABLE TRAITS, DISEASE SUSCEPTIBILITIES, AND INBRED STRAINS

Gene targeting is best used to study genes that have already been linked to human disease, but targeting techniques are of limited utility in the discovery of *new* health-related genetic factors. How can we come to identify and understand the functions of the remaining genes? Many disease-related genes have been efficiently tracked in human families, especially genes associated with inherited diseases that are expressed in a simple yes–no fashion, muscular dystrophy, cystic fibrosis, and a small fraction of breast and colon cancers. Those kinds of genes will be most easily and efficiently identified through studies of human patients and human DNA. But many of the genes that are most important to everyday health produce disorders that are more subtle and more variable and whose expression depends heavily on genetic background or environmental influence. These are the genes that are most difficult to study in the human population; but they are relatively easy to study in mice.

One important key to the successful use of mice as genetic models is the existence of stable, well-characterized inbred strains.[1] Essentially, inbred strains can be considered as large collections of genetically identical twins. Animals of 2 inbred strains can be quite different from each other, exhibiting different toxin sensitivities, reactions to drugs, disease susceptibilities, and so on. For example, when an experiment is conducted with a group of animals of the C57BL6 strain, genetic variation need not be considered as a factor contributing to the variability of the experiment; the genetic components of a trait will not vary between the individual animals. However, if the same experiment is performed with animals of the 129/Sv strain, consistent differences in response between the 2 C57BL6 groups can be attributed to genetic differences, with a considerable degree of certainty. If all members of a particular inbred strain are fed the same mouse chow, drink the same water, and are housed in similar cages in the same building, the effects of environmental exposure can be minimized. This kind of consistency, which is so crucial to analysis of genetic and toxicity data, cannot be achieved with most animal models, and it certainly is not possible in the human population.

The genetic and physiologic differences between individuals in various inbred strains have been used to track the genetic locations of a number of quantitative-trait loci (QTLs), contributing to phenotypic characteristics as varied as tumor susceptibility, obesity, drug preference, and tendency to addiction.[2,3] The QTL alleles contribute

to these and other types of disorders not in an independent yes–no fashion, but in a concerted, generally additive manner. Those factors can be studied more easily with the fixed genetic backgrounds of specific inbred strains. But can these mapped mouse QTL alleles and mutant phenotypes be used with any certainty to help us to identify human genes with similar effects? How similar *are* mouse and human genes?

EXTRAPOLATING GENETIC INFORMATION FROM HUMAN TO MOUSE AND BACK AGAIN

After years of genetic research, we understand a considerable amount about the structure and genetic organization of mouse chromosomes. As genetic studies have demonstrated, mouse and human chromosomes are similar in gene content and gene organization, with a few important exceptions. To understand how the 2 genomes are related, first imagine that 23 pairs of human chromosomes were shattered into many bits of different sizes—200–300 bits ranging in size from 50 to 500 genes. Next, imagine that those 200–300 bits were reassembled into new structures without the benefit of a map or key to tell us which pieces went with which. Relative to the human genome, mouse chromosomes appear to be assembled in just this way.[4] In general, genes have not been added or deleted; there is nearly a 1-to-1 correlation between the genes in mouse chromosomes and the genes in related regions of human chromosomes. In other words, nearly all human genes have a direct mouse counterpart. I say *nearly* because there are indeed examples of human genes that do not have a direct functional equivalent in mice; there are also mouse genes with no known human gene cognate.[5] Most of the different ones are of a particular type: recently evolved members of gene families that arose from repeated duplications of a single ancestral gene and that were modified by genetic drift and gene conversion. The recently evolved gene-family members might be particularly interesting because in general they represent the acquisition or loss of unique gene function. These "extra" or "missing" genes might therefore be the most-easily traced clues to species-specific differences in development, basic metabolism, disease susceptibilities, and other characteristics.

Within most of the 200–300 lists of chromosomes, however, mouse and human regions contain related genes organized in a very similar manner—in the same order along the chromosome, for example, and at about the same distances from one another.[5–7] This genetic similarity provides us quite a lot of predictive power. Because the maps of related mouse and human regions are, in many senses, mirror images of each other, genetic data, DNA sequence data, and other information can be extrapolated from human regions into mouse. Similarly, genetic and physiologic data derived from the analysis of mouse mutations can be used to predict the existence and locations of human genes with specific biologic functions.

For example, consider the structure of human chromosome 19q (as has been mapped by members of the Lawrence Livermore National Laboratory's Human Genome Center[8]) aligned with the homologous region of proximal mouse chromosome 7. Close examination shows that pairs of related mouse and human genes are found in more or less the same order, and with nearly identical spacing, along the lengths of the 2 related chromosomal regions.[7] Because gene content and order have been so well preserved, we can predict with confidence that a new gene mapped to chromosome 19q will have a counterpart in mouse and that it will be in a specific region of chromosome 7. In addition, every new mouse mutation that is assigned to this region of mouse chromosome 7 can be predicted to define a functionally similar transcription unit in human 19q. With a clear understanding of the limitations, we can use the clone resources, mapping data, and sequencing information being collected for human 19q as though they were derived from the related region in the mouse. In many senses—and with caution—the mouse and human genomes can therefore be studied in parallel as 2 highly divergent versions of the same gene set.

SPONTANEOUS AND INDUCED MOUSE MUTATIONS TO PROVIDE A LARGE AND VARIED SELECTION OF HUMAN-DISEASE MODELS

Variety Is the Key: Many Ways to Make a Model Mouse

What kind of mutant resources are available for analysis of acquired and inherited human disease? A remarkable number of known, mapped mouse genetic mutants and variants have been generated and collected over

the last several decades, and well-developed techniques are available for adding to this valuable mutant resource. Transgenic techniques are among the more-recently developed means of deriving new mouse mutants. But most existing mouse models have been generated by more-traditional mutagenesis methods: through exposure of animals to mutagens, such as radiation and chemicals. Many of the existing mouse mutants arose as byproducts of risk-assessment studies, a major focus of efforts funded by the Department of Energy over the past 40 yr. For example, William and Liane Russell and colleagues at Oak Ridge National Laboratory have played a dominant role in establishing the guidelines for radiation and chemical exposures of humans and have done so by monitoring numbers of mutant offspring produced in radiation- and chemical-treated mice.[9,10]

A number of the mutant offspring that arose from those mutagen-treated parents expressed disorders that resembled various types of human disease, and the Russells and co-workers wisely saved the mutant animals for future genetic studies. Many other mouse mutants, generated in similar risk-assessment studies in other institutions or arising spontaneously in large breeding colonies, are now the subjects of research carried out by molecular biologists, pathologists, embryologists, and others in institutions around the world.[11] These mutants were selected from the animal colonies because they expressed specific types of health-related disorders, and not because of a priori knowledge regarding the nature of the genes involved. Although it can be difficult to link such "randomly generated" mutations to defects in specific genetic loci, the process is becoming less and less difficult as the results of the Human Genome Project begin to unfold and as new information about genome structure, gene location, and gene function begins to flood the public databases. The genes that are found linked to the randomly generated mutations are often novel or are known genes that would not have been expected to be associated with the types of disorders seen in the mutant mice. Therefore, spontaneous, induced, and targeted mutations constitute highly complementary tools for discovering links between genes and hereditary disease. The power of the mouse is its versatility: Targeted mutations can be induced in known genes that are thought to be required for health, and new genes, or more-subtle effects of known genes, can be discovered through analysis of randomly placed mutations that are selected specifically for their phenotypic effects.

Unlike targeted mutations, the genetic defects that arise spontaneously or as a result of mutagen treatments are of many different types—not just "knockouts," but also missense and regulatory mutations that permit the expression of a gene at different levels, in different tissues, or in forms slightly modified from those seen in normal mice. The mutations can alter the function of the protein product in subtle, but nevertheless insidious, ways. Missense mutations, which cause substitution of a single amino acid in an otherwise normal protein, can sometimes produce severe health effects, whereas complete absence of the same protein, as would be caused by a gene knockout, might be relatively benign.[11,12] In other cases, where absence of a protein proves lethal, relatively mild missense mutation can provide a means of studying the effects of a gene defect in live-born mice.

CAN GENE'S HEALTH-RELATED FUNCTIONS BE DEDUCED FROM THE PHENOYTPES OF MUTANT MICE?

Are genetic defects expressed by a mouse truly good indicators of the genes that are critical to human disease and susceptibilities? Certainly there are biologic differences between rodents and humans that prevent mice from serving as perfect models. However, a number of related mouse and human gene pairs are known to perform similar or even identical biologic functions. For example, defects in the mouse *Kit* gene cause pigmentation, hematopoietic, and reproductive defects associated with a classical mouse mutation called dominant white spotting, or *W*. The relationship between *Kit* and *W* was originally discovered when the human gene, KIT, was mapped to the centromeric region of human chromosome 4. This region is known to be related to the *W* region of mouse chromosome 5; because the gene's properties suggested a link to hematopoietic cell growth, *Kit* was immediately suspected as the "culprit" gene responsible for the phenotype of *W* mice. The proof of the genetic connection was established soon after.[13,14]

In fact, the pigmentation defect seen in *W* mice is almost identical to that observed in people who inherit what is called the piebald trait, and some of these people, but not all of them, have since been found to carry mutations in the human *Kit* gene.[15] Piebald trait had been difficult to map in human families because, as in mice, a similar type of pigmentation pattern can be caused by mutations in several genes. Establishing the connection between *Kit*

and *W* provided the essential link that permitted the genetic cause of one type of piebald trait to be discovered in human patients, a feat that could not be accomplished directly through studies of human families. Since then, several other links between genes and human disease have been established first through analysis of mutations that cause similar symptoms in mice. A recent example is the link between the leptin gene and obesity in *ob* mutant mice.[16] Although such mutations are rare, obese human patients with leptin gene defects recently have been identified,[17] providing the only certain link between a single gene and obesity in humans. Genes linked to specific types of human deafness,[18] neural-tube defects,[19] and other defects have also been discovered through the isolation of genes linked to similar, more easily mapped mouse mutations. As this trend continues, it is likely that more and more human-disease genes will be first discovered in mice.

An Unmet Challenge: Tracing the Causes of Human Birth Defects

One of the remaining unmet challenges in human-health research is in the discovery of the causes of human birth defects. Although some birth defects are inherited, the bulk of human developmental disorders are not strictly, or even primarily, genetic; most are probably due to a combination of genetic susceptibilities and environmental factors. I use the word probably because we can only guess at the causes of most common developmental defects. Most human birth defects arise without warning, without the benefit of diagnosis and prenatal counseling, and without any credible medical explanation.

Because the mouse has long been used as a model for human development, the existing mutant collections are especially rich in animals that have various types of prenatal defects.[11,20] Although the abnormalities that are seen in most human infants born with birth defects—such as limb deformities, skeletal malformations, neurologic disorders, and hydrocephalus—are not mainly genetic, the genes that are disrupted in animals with similar disorders can tell us a considerable amount about the potential causes of those human defects. What kinds of proteins are essential to the normal process of limb, skeletal, neural-tube, or nervous-system development? With which molecules might those essential proteins interact, and what kinds of molecules might interrupt their function? The same proteins that are disturbed by genetic defects—or other proteins that are part of the same developmental pathways—are likely also to be inhibited, stimulated, or altered by drugs, exposures, or environmental toxicants. Pinning a development function to a specific mutated gene therefore might not directly identify the factors that are key to genetic susceptibilities, but it does provide new tools that can be used to gain molecular access to the most-sensitive prenatal events.[20]

SUMMARY AND CONCLUSION

The mouse has been studied as a model for many years, but the use of the mouse in analysis of human-health disorders—from outright genetic disease to cancer susceptibility, developmental disorders, quantitative traits, and other problems influenced by genetic background and environmental exposures—is just beginning to reach full potential. As new mutants are created and the human and mouse genomes are further linked through gene mapping, gene sequencing, and basic gene-expression studies, the mouse is certain to become a major tool for linking mapped human genes to biologically relevant, in vivo functions. The combined power of targeted and induced mutagenesis methods, together with well-controlled methods for studying the influence of genetic background and environment on the expression of traits in normal and mutant animals, makes the mouse a unique and valuable resource for the dissection of complex human traits.

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Part III

Protecting the Environment

Environmental Research

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The next group of papers discusses the environmental side of the Biological and Environmental Research (BER) program, and a few comments here will set the context.

Over the last 50 yr, the treatment of the environment in the BER program, like the treatment of the environment in most federal research programs, experienced a paradigm shift. The shift was away from the perception that the environment was simply the medium by which various insults, whether chemical or radiation, reached humans to the view that the environment is the ultimate recipient of those insults. We have seen many examples of that shift.

The BER program has done much important work and made major contributions to the understanding of many environmental problems—the invention of radioecology by Eugene Odum at the Savannah River Laboratory in the early 1960s, the first attempts to develop climate models by Chuck Leith at the Lawrence Livermore National Laboratory, the seminal work that Warren Washington did at the National Center for Atmospheric Research, the studies of the fallout of radioactivity from various weapons tests, the theoretical and practical studies dealing with turbulent diffusion and transport by Frank Gifford at the Oak Ridge Atmospheric Turbulence and Diffusion Laboratory, the studies of long-range transport of plumes from power plants and the early work dealing with the acid-precipitation problem, and the launching of the first federal research program 20 yr ago to look at the greenhouse effect, which at the time was known only to very few scientists and science managers. It also added its unique twist and its unique culture to the milieu of the federal agencies and the scientists who attacked many of those problems. The program sometimes stood alone in the midst of various interagency battles, but more often it was an essential and serious team player in interagency efforts. The BER program has contributed uniquely in promoting environmental science and in bringing environmental sciences to bear in the solution of national problems.

One unique characteristic of the program is its position in the Office of Energy Research and the Department of Energy (DOE). Where you stand on an issue depends on where you sit. The BER program is one example of that saying, and because of its position in the high-powered, big-time physics environment in which it exists in DOE, the program has managed to aim many of the physics capabilities, such as remote-sensing technologies and high-performance computing, at environmental problems that have eluded other agencies. Also, it has brought a culture of multidisciplinary science to the solution of environmental problems through its ability to launch teams of investigators, bringing together laboratory and university scientists to address problems. We are proud of the culture that we have applied to many environmental problems.

Atmospheric Carbon Dioxide: Contemporary Budget, Historical Context, and Implications for the Future

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Carbon dioxide (CO_2) produced by the burning of fossil fuel—coal, oil, and natural gas—is the largest waste product of modern industrial society. Emissions to the atmosphere globally in 1997 exceeded 6 billion tons, more than 1 ton for every person on the planet. The United States, with about 5% of the world's population, was responsible for 22% of global emissions. The People's Republic of China, which ranked number 10 in 1950, is now number 2, having just surpassed the former Soviet Union. Given current trends, there is little doubt that China will soon be number 1 and will set the pace globally for CO_2 in the future.

HISTORICAL CONTEXT

We have an excellent account of variations in the concentration of atmospheric CO_2 over the last 160,000 yr. The modern instrumental record, based on sampling of air at remote sites around the world, dates to 1958. The continuity and quality of the modern data for Mauna Loa, Hawaii, and the South Pole, displayed as weekly averages of daily measurements in figures 1 and 2, are tribute to the ingenuity and skill of C.D. Keeling at the Scripps Institute for Oceanography, who, long before it was popular, saw the need for a precise record of variations in atmospheric CO_2.[1] Studies of gases trapped in polar ice, pioneered by Swiss and French teams of scientists led by Hans Oeschger and Claude Lorius, extend the record back in time and indicate that the modern rise in CO_2 began in the early part of the 18th century.

A summary of the ice-core data is presented in figures 3 and 4.[2] The abundance of CO_2 was relatively constant, at about 280 parts per million by volume (ppmv) of dry air, for more than 10,000 yr after the end of the last ice age. It rose from 280 ppmv in the 18th century to about 315 ppmv in 1958 and has since continued to climb to a contemporary value close to 360 ppmv. Concentrations of CO_2 were much lower, about 200 ppmv, during both the last and the penultimate ice ages, which ended about 15,000 and 125,000 years ago, respectively.

The modern rise, as we shall see, may be attributed to complex contributions from industrial and agricultural society: Emission of CO_2 associated with combustion of fossil fuel played a major role, but that was not the only influence. At least a portion of the modern increase must be attributed to transfer of carbon to the atmosphere from soils and the terrestrial biosphere. A particular challenge, if we are to forecast the future course of CO_2, is to differentiate between contemporary contributions from fossil fuel and the soil-biosphere system.

Thanks mainly to work sponsored by the Department of Energy, we have a reasonably accurate estimate of the quantity of CO_2 added to the atmosphere in recent years by the burning of fossil fuel. With somewhat lower precision based on historical data, trends in emission can be reconstructed back to the middle of the 19th

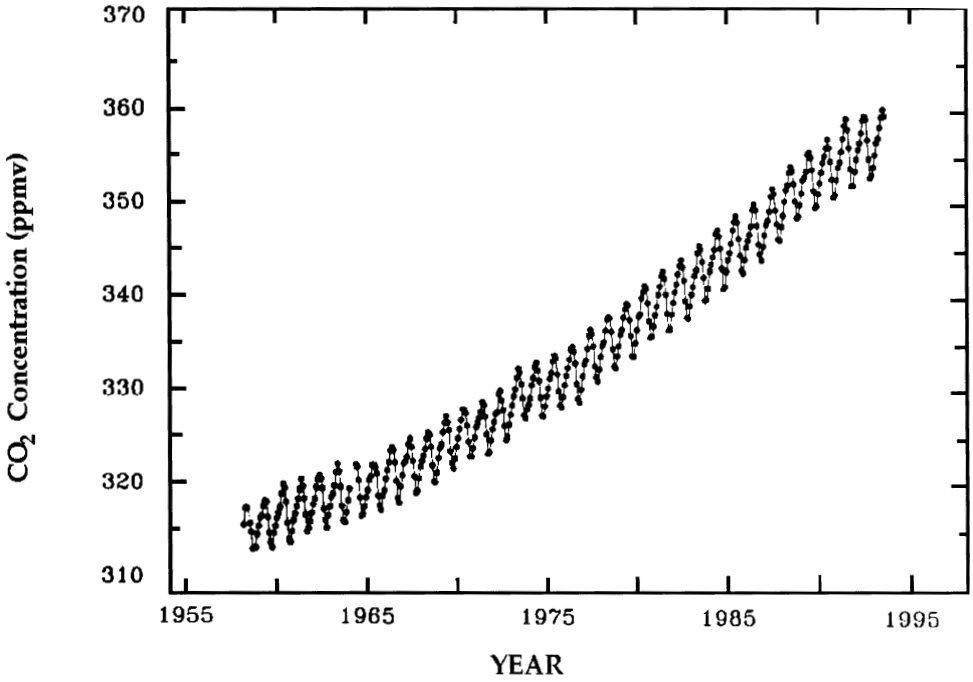

FIGURE 1 Monthly atmosperhic CO_2 concentrations at Mauna Loa (Keeling and others 1994).[1]

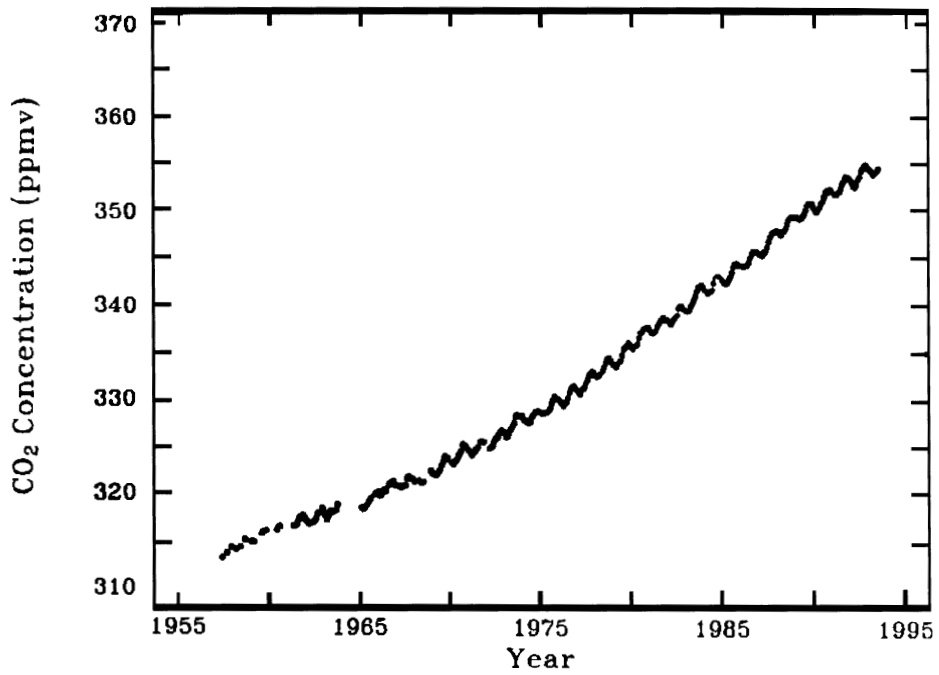

FIGURE 2 Monthly averages of the concentration of CO_2 at the South Pole (Keeling and others 1994).[1]

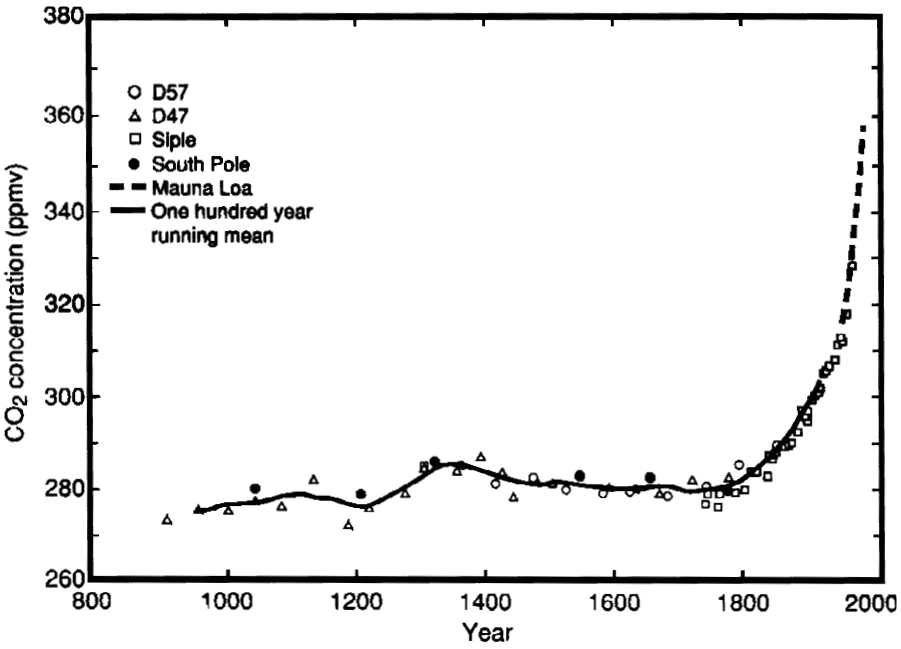

FIGURE 3 CO_2 concentrations over the past 1,000 years from ice-core records (D47, D57, Siple, and South Pole) and (since 1958) from Mauna Loa, Hawaii measurement site. The smooth curve is based on a hundred year running mean. The rapid increase in CO_2 concentration since the onset of industrializaiton is evident and has followed closely the increase in CO_2 emissions from fossil fuels (adapted from Climate Change 1994[2]).

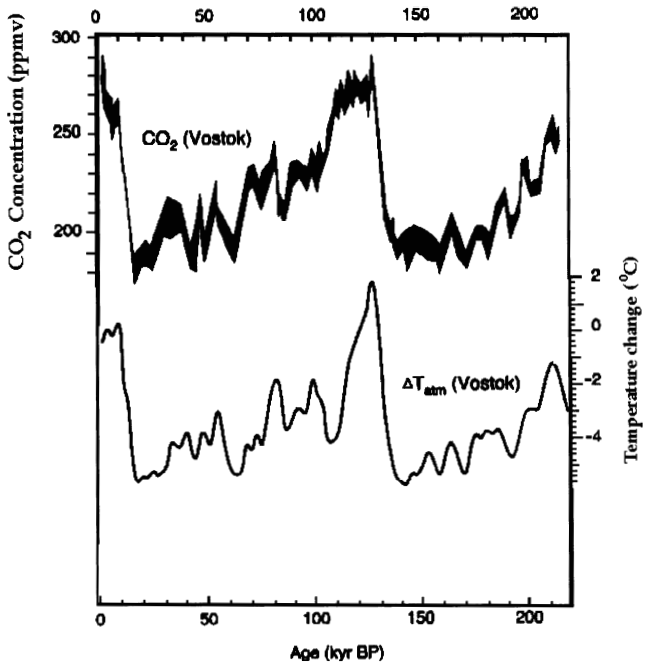

FIGURE 4 Temperature anomalies and CO_2 concentrations over the past 220,000 yrs., as derived from the ice core record at Vostok, Antarctica (adapted from Climate Change 1994[2]).

century. Results of this analysis, adapted from C.D. Keeling[1] and Marland and co-workers,[3] are presented in figure 5. As the data indicate, until recently, combustion of coal was the major industrial source of CO_2. Oil supplanted coal as the largest source of CO_2 in the late 1960s and the contribution from natural gas has been increasing in recent years.

Per unit of energy delivered, coal is the largest source of CO_2, followed by oil and gas. In 1991, oil accounted for 37.1% of global consumption of commercial energy, coal 29.2%, and gas 23.7%, with the balance attributable to nuclear (7%) and hydroelectric (3%). The relative contributions of oil, coal, and gas to industrial emissions of CO_2 in the same year were 42%, 38%, and 17%, respectively; the balance was associated with gas flaring and the manufacture of cement.

This paper seeks to develop a consistent model for the contemporary budget of atmospheric CO_2 . Emission of CO_2 from industrial sources from July 1991 to July 1994 would have been sufficient to increase the concentration of atmospheric CO_2 by 8.85 ppmv.[4] The observational data indicate that the rise in CO_2 over the interval was only about one-third of that value, 2.89 ppmv. One would expect that a portion of the CO_2 added to the atmosphere was transferred to the ocean. Emission, or uptake, of CO_2 associated with a change in the global content of carbon in soils and the terrestrial biosphere (defined here as the composite of all living, land-based plant material, including both roots and above-ground components) has an additional influence, complicating attempts to develop a balanced budget for CO_2 in the atmosphere. I outline an approach using measurements of changes in the abundance of atmospheric O_2 and the isotopic composition of CO_2, allowing in principle for a separation of the relative contributions of industry, the soil-biosphere system, and the ocean to the budget of atmospheric CO_2. I conclude with summary remarks discussing implications for policy and opportunities for further work.

THE CONTEMPORARY BUDGET OF ATMOSPHERIC CO_2

We know that at least part of the modern increase in CO_2 can be attributed to emissions associated with combustion of fossil fuel. We know that a portion of the carbon emitted by burning fossil fuel will remain in the atmosphere and that some fraction will be taken up by the ocean. A major uncertainty concerns the importance of exchange between the atmosphere and the combination of the terrestrial biosphere and soils. Net exchange of

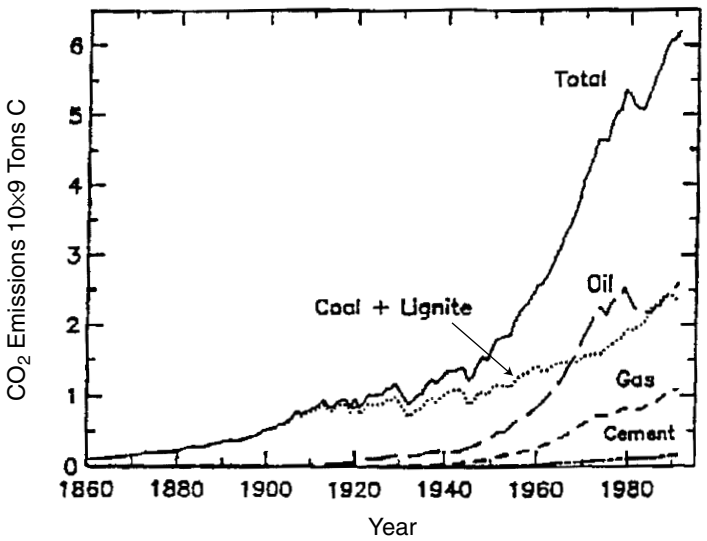

FIGURE 5 Production of CO_2 associated with the combustion of fossil fuel and cement production (adapted from Marland and others[3]).

carbon between the biosphere-soil system and the atmosphere reflects the composite influence of human activity on the global biosphere. Experience in the United States and Europe indicates that this influence can be extremely complex and difficult to quantify. In principle, the biosphere-soil system could represent either a source or a sink for atmospheric CO_2 on a global basis. Compounding the problem, it could provide a source in 1 geographic region, a sink in another.

Deforestation in the tropics constitutes a source of CO_2. But regrowth of forests in regions previously deforested should contribute a sink. A warmer climate could promote growth of vegetation (by prolonging the growing season, for example) and thus produce a sink for CO_2. Offsetting, higher temperatures could enhance decomposition of organic matter in soils, resulting in a net source of CO_2. Higher concentrations of CO_2 and industrial sources of fixed nitrogen and sulfur could contribute to increased growth of vegetation, representing a sink for CO_2. But the carbon-to-nitrogen ratio in plants could change in response to a change in the availability of these essential elements, and feedbacks in terms of exchange of carbon with the atmosphere could be altered accordingly. These influences are complex and difficult to forecast. We need to develop a sense of the factors that regulate exchange of carbon between the biosphere-soil system and the atmosphere today if we are to project how they might vary in the future.

It is clear that the biosphere-soil system was an important net source of CO_2 in the past. The beginning of the modern increase in CO_2 in the 18th century predates important input from combustion of fossil fuel, and this situation persisted until the beginning of the 20th century. The carbon content of the atmosphere increased by about 15 billion tons between 1860 and 1890 (corresponding to a rise in the mixing ratio of CO_2 by 7 ppmv), as indicated by the ice-core data. Combustion of fossil fuel over the same period contributed 5.8 billion tons of carbon as CO_2, too small to account for the observed buildup of CO_2 (the discrepancy is even larger if we allow for the fact that less than 60% of the fossil source would be expected to persist in the atmosphere).

It should come as no great surprise that 100 yr ago transfer of carbon from the biosphere and soil to the atmosphere was a larger source of atmospheric CO_2 than was combustion of fossil fuel. Where it was available, wood was a convenient and relatively inexpensive fuel for use both in domestic situations (for heating and cooking) and in industry, where it was used extensively not only as a fuel but also as a source of essential industrial feedstock, supplying, for example, the large quantities of charcoal consumed in smelting ore. As forests were depleted in the early part of the 18th century, coal replaced wood as the primary fuel used in England and in much of western Europe. The transition from wood to coal occurred almost 200 yr later in the United States, in the early part of the 20th century. It is an even more recent phenomenon in China and India. As fossil fuel substituted for wood in developed societies, forests were often allowed to regrow. In the eastern United States, for example, forests were destroyed in the 18th century to provide wood for industry and land for agriculture. Construction of the railroads in the 19th century provided access to much-richer agricultural land in the Midwest. Plowing the relatively pristine prairies of the Midwest would have stimulated release of CO_2, as organic matter that had been deposited over millennia was exposed to atmospheric oxygen. Farming in the East declined as a consequence of the more-efficient supply of agricultural products from the Midwest. Land was abandoned and allowed to revert slowly to forest. As we will see, it is likely that forests and soils in the eastern United States are now probably a net sink for atmospheric CO_2.

Modern agricultural practices provide an important opportunity to enhance retention of carbon in soils—to capture at least a portion of the carbon released from soils in the early days of agricultural exploitation. Results from 27 studies primarily in the United States and 17 similar investigations in Canada indicate that soils subjected to nontill practices were more effective in retaining carbon than soils cultivated with conventional practices. Additional storage ranged from –4 to +10 Mg/ha with a mean of +3 Mg/ha. If nontill practices were instituted on a large scale in the United States and Canada, it is estimated that over a 20-yr period 765 teragrams of additional carbon could be sequestered in soils, significant in absolute terms but small compared with the fossil source.[15]

It would be helpful to know what is happening today, if only on a global scale. If we could provide spatial information, even on a coarse scale, this would be a bonus and an incentive for further, more-directed research. This paper describes an approach that promises at least to address these objectives. The architect and first practitioner of the approach is another Keeling: R.F. Keeling, son of the C.D. Keeling cited earlier for his contributions to modern studies of CO_2. It is based on a recognition that careful measurements of the abundance

of O_2 in the atmosphere, coupled with measurements of CO_2, can provide a means to distinguish between the roles of the ocean and biosphere-soil system as a sink for fossil-fuel-derived CO_2. While a graduate student at Harvard University, R.F. Keeling developed an instrument with the capability to measure the abundance of O_2 to the precision required to implement this strategy and demonstrated its potential in an important series of papers analyzing implications of the changes in O_2 that have occurred over the last several years.

Combustion of fossil fuel is responsible both for an increase in production of CO_2 and simultaneously for a (predictable) decrease in O_2. The relative decrease in O_2 is largest if the fuel consumed is natural gas (CH_4), less for oil, and least for coal, reflecting the carbon-to-hydrogen ratios of the different fuels. On the average, with the current mix of fossil fuels, accounting for production of cement, it is estimated that 1.4 moles of O_2 is removed from the atmosphere for each mole of CO_2 released by the burning of fossil fuel. Independent analysis suggests that uptake of CO_2 by the biosphere (photosynthesis) is associated with release of O_2 in relative proportions of 1 mole of CO_2 for each 1.1 moles of O_2, proportions are assumed to be similiar for release of CO_2 and consumption of O_2 by the biosphere and soils (the combined effects of respiration and decay). The net global source of CO_2 (the composite release due to transfer from the biosphere-soil system and burning of fossil fuel) will be distributed between the atmosphere and ocean in proportions that depend on the capacity of the ocean to absorb excess CO_2, measured in terms of a quantity known as the airborne fraction or Keeling fraction.[5] The change in O_2, however, is essentially confined to the atmosphere, reflecting the relatively low solubility of O_2 in water. Given a measurement of the change in O_2 and knowing the change expected because of combustion of fossil fuel, we can estimate the contribution of oxidation (or reduction) of biosphere-soil organic carbon to the observed change. Given a measurement of the change in the abundance of atmospheric CO_2 and information on the contribution of burning of fossil fuel and oxidation of organic carbon in soils and the biosphere to this change, we can obtain empirical estimates of the quantity of carbon transferred from the atmosphere to the ocean and of the net exchange of atmospheric carbon with the biosphere and soil.

Changes in O_2 are quoted conventionally in terms of the fractional change in the ratio of the concentration of O_2 to N_2 with respect to the ratio in a standard (typically a sample of air taken in 1988 when R.F. Keeling began his program of sustained modern measurements of O_2). The change in O_2 relative to N_2 is expressed in terms of the delta notation.[4] Assuming that the concentration of N_2 stays fixed, a change in the mixing ratio of O_2 relative to N_2 by 1 ppmv corresponds to a change in delta of 1/0.2095 = 4.77 per meg (0.2095 is the mixing ratio assumed for O_2 by volume in dry air).

Emission of CO_2 from combustion of fossil fuel from July 1991 to July 1994 would have been sufficient to cause an increase in the mixing ratio of CO_2 by 8.65 ppmv. The equivalent decrease in delta for O_2 would be given by (8.65)(1.39)(4.77) = 57.4 per meg. The observed decrease is 42.2 per meg. It follows that exchange of O_2 between the biosphere-soil system and the atmosphere must be responsible for a net increase in delta of 15.2 per meg. That implies that the biosphere-soil system accounted for a net source of O_2 over this period, and thus for a net sink of CO_2. The equivalent sink for CO_2 (converting the change in delta to an equivalent change in the mixing ratio of O_2 and accounting for the fact that 1.1 mole of O_2 is released for every mole of CO_2 taken up by the biosphere-soil) would be given by [(15.2)(0.2095)]/(1.1) = 2.89 ppmv. The observed increase in CO_2 is 3.36 ppmv. The ocean must have accounted for removal of CO_2 equivalent to (8.65 – 2.89 – 3.36) = 2.4 ppmv.

A summary of this analysis is presented in figure 6. Point A denotes the (CO_2, O_2) combination measured at the beginning of the record, in July 1991. Point D summarizes observations for the end of the record, in July 1994. The influence of fossil-fuel combustion (production of CO_2 and consumption of O_2) is represented by the segment A-B (slope, –0.15 ppmv meg). The role of the ocean, providing a sink for CO_2 with minimal impact on O_2, is indicated by the horizontal line B-C. Finally, the path from A to D is completed by exchange between the atmosphere and the biosphere-soil system as described by segment C-D (slope, = –0.19 ppmv meg). Point C lies at the intersection of a horizontal line passing through B and a line of slope –0.19 ppmv meg passing through the final state D.

In summary, from the middle of 1991 to the middle of 1994, according to R.F. Keeling and co-workers,[4] we added enough CO_2 to the atmosphere from combustion of fossil fuel to increase the atmospheric abundance by 8.65 ppmv—about 18.3×10^9 tons of carbon. Of that, a quantity of CO_2 equivalent to 2.89 ppmv (6.13×10^9 tons of carbon) was incorporated either in soils or in the terrestrial biosphere, 2.39 ppmv (5.07×10^9 tons of carbon)

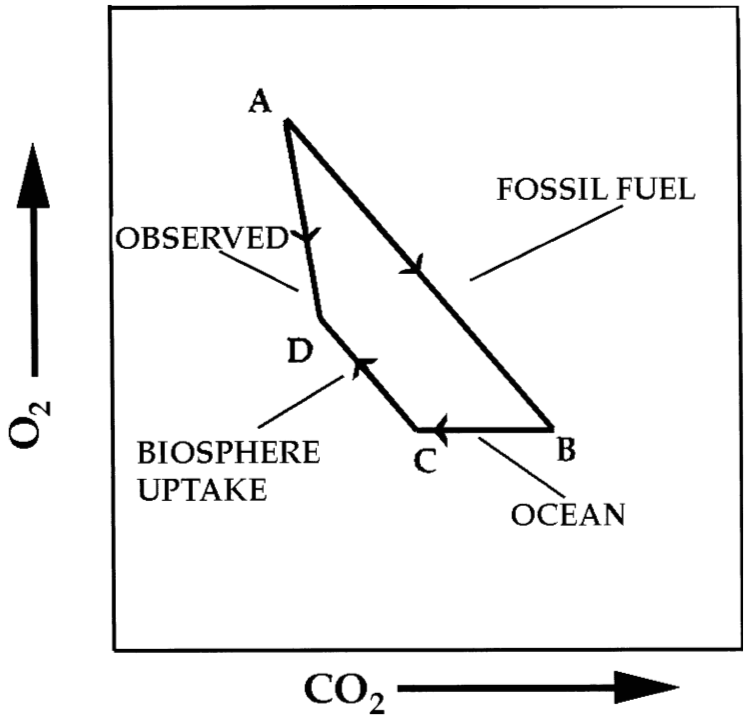

FIGURE 6 Schematic illustration of trends in CO_2 and O_2, 1991-1994.

was transferred to the ocean, and 3.36 ppmv (7.12×10^9 tons of carbon) remained in the atmosphere. Of the carbon added to the atmosphere by the burning of fossil fuel, 39% remained in the atmosphere, 33% was incorporated in a combination of soils and the biosphere, and the balance, 28%, was absorbed by the ocean. The analysis implies that 58% of net carbon added to the atmosphere (fossil-fuel source minus biosphere-soil sink) over the period 1991–1994 remained in the atmosphere and the balance was transferred to the ocean. On a global basis, it appears that in the early years of the 1990s the carbon content of soils and the terrestrial biosphere increased at an annual rate of close to 2×10^9 tons per year.[4]

From an analysis of gradients in CO_2 and O_2 observed between the Northern and Southern hemispheres, R.F. Keeling and associates[4] concluded that the growth of the biosphere-soil reservoir in the 1990s occurred primarily in the north. Transfer of air between the Northern and Southern hemispheres is relatively slow. The associated time constant—as indicated, for example, by analysis of spatially distributed data for the industrial halocarbons—is about 1 yr. Fossil fuel is consumed mainly in the industrial Northern Hemisphere. If net input of CO_2 to the Northern Hemisphere was dominated by fossil fuel, we would expect the abundance of CO_2 in the atmosphere of the Northern Hemisphere to exceed that in the Southern Hemisphere by about a year's worth of production. The abundance of O_2 would be correspondingly lower in the north. The observations indicate that the gradient of concentrations between the hemispheres is less than one would expect according to that scenario. The dilemma is resolved if we suppose that the net input of CO_2 (and net consumption of O_2) in the Northern Hemisphere was less than would have been expected as a consequence of fossil-fuel consumption. The apparent reduction in the net northern CO_2 source (and the smaller decrease in O_2) can be explained if the global biosphere-soil sink for CO_2 (source of O_2) inferred from the CO_2–O_2 analysis is associated primarily with growth of the biosphere-soil system in the north.

Direct (in situ) measurements of CO_2 and O_2 sufficient to carry out the analysis of the relative importance of the ocean and the biosphere-soil system in the global budget of atmospheric CO_2 are available only for the 1990s.

In a remarkable tour de force, a group of scientists from the University of Rhode Island, Pennsylvania State University, the National Oceanographic and Atmospheric Administration, and the University of Colorado[6] extended the study by R.F. Keeling and co-workers[4] back to 1977 by measuring the composition of air in the upper unconsolidated (firn) layer of snow at the South Pole. Analysis of the firn data indicates that the biosphere-soil system played a much smaller role in the global budget of atmospheric CO_2 over the period 1977–1985 than it did in the 1990s; trends in CO_2 and O_2 observed in the earlier data can be attributed straightforwardly to the influence of fossil-fuel combustion without invoking a role for either the biosphere or soil. It appears, therefore, that the

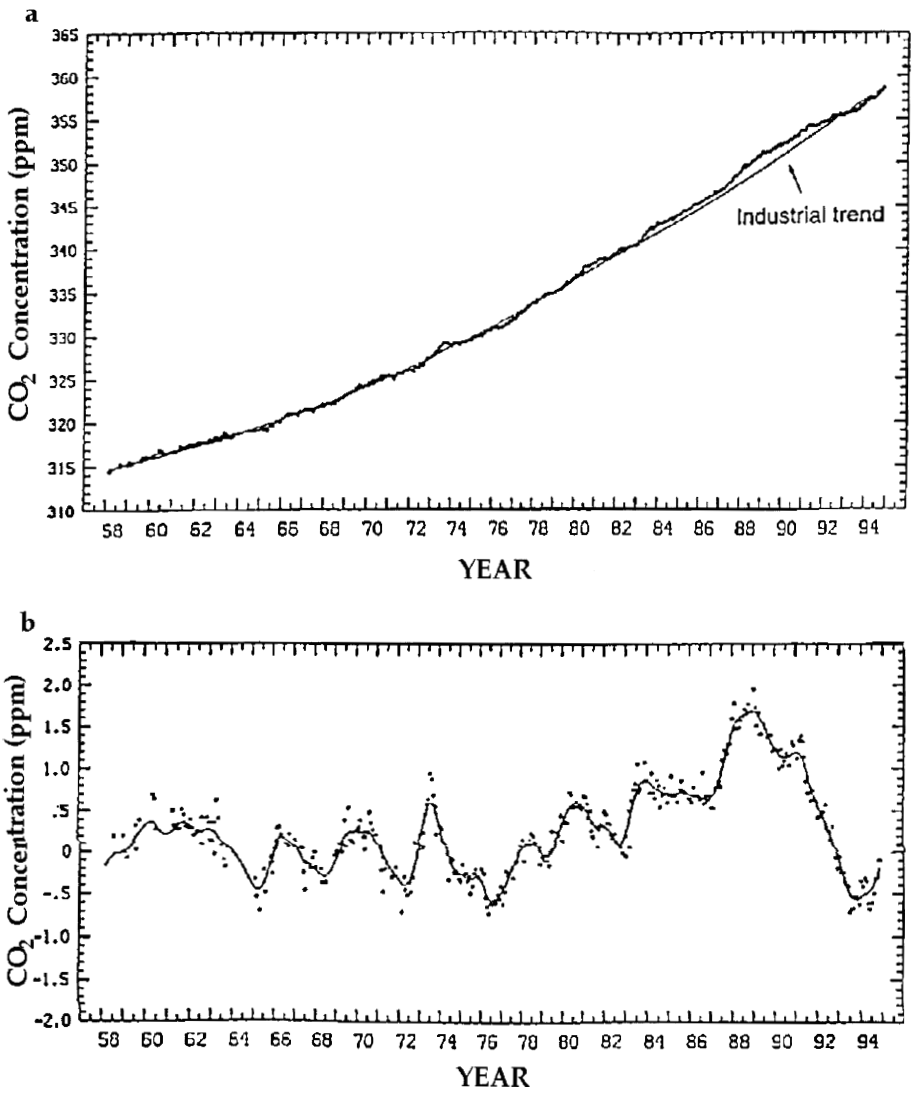

FIGURE 7 (a) The long-term trend in the abundance of atmospheric CO_2 obtained from an average of data from Mauna Loa, Hawaii, and South Pole after seasonal adjustment of monthly averages at both stations. The curve labeled "industrial trend" was derived with an assumption that combustion of fossil fuel and cement manufacture were primarily responsible for changes in CO_2 and that 55.9% of emissions persist in the atmosphere. (b) The anomaly in CO_2 obtained by subtracting the industrialized trend curve in (a) from the observed, monthly mean, CO_2 observations (adapted from Keeling and others, 1995).

biosphere-soil system played a minor role in the global CO_2 budget in 1977–1985 even though it was clearly important in the 1990s.

An interesting perspective on the factors influencing changes in the abundance of CO_2 since 1958 was presented in a paper published by C.D. Keeling and associates[7] in 1995. As indicated in figure 7, they found that the trend in CO_2 from 1958 to 1980 could be explained if fossil-fuel combustion was the dominant source of CO_2 and the airborne fraction was taken as 56%. The airborne fraction that they used is almost identical to the value (58%) inferred from the CO_2–O_2 observations for 1991–1994 when we allow in the latter case for uptake of carbon by the biosphere-soil system. Assuming that the capacity of the ocean to take up excess CO_2 is relatively constant, the obvious conclusion to be drawn is that the role of the global biosphere-soil system, although important in the 1990s, must have been small throughout the period 1958–1985; the conclusions of C.D. Keeling and co-workers[7] are consistent with those of Battle and co-workers.[6]

There is a simple resolution to the dilemma. The gradient in CO_2 between the Northern and Southern hemispheres throughout the record is consistently less than would be expected if input from combustion of fossil fuel were the only factor influencing this gradient. Moreover, as indicated in figure 8, the gradient appears to increase in proportion to the source of CO_2 contributed by fossil fuel. We can account for all the existing constraints if we assume a persistent sink for CO_2 associated with uptake of carbon by the biosphere-soil system at the middle latitudes of the Northern Hemisphere. The analysis of R.F. Keeling and co-workers[4] suggests that this sink contributed to a net annual removal of about 2×10^9 tons of carbon from the atmosphere in the early 90s—roughly one-third of the input from fossil fuel. For most of the time, the middle-latitude Northern Hemisphere sink is offset by a source of CO_2 of comparable magnitude in the tropics, probably attributable to deforestation in some countries, including Brazil and Indonesia. The tropical source appears to have been much reduced in the early 1990s in the period covered by the observations of R.F. Keeling and co-workers.[4] Indeed, there are data to support that conjecture. Burning of forests in Brazil appears to have diminished in the early 1990s largely because of changes in economic incentives, helped perhaps by sensitivities heightened by Brazil's role in hosting the Earth

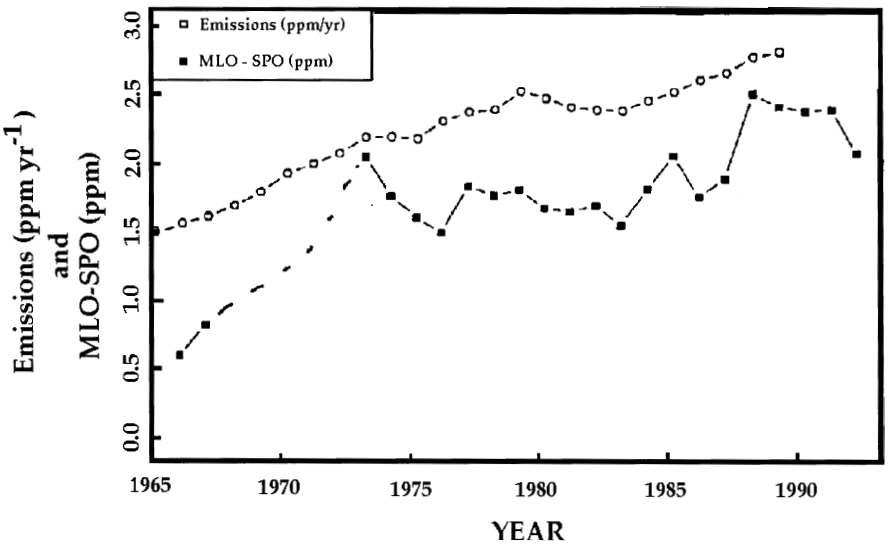

FIGURE 8 Emissions of CO_2 associated with combustion of fossil fuel (o), quoted in terms of the equivalent change in the abundance of atmospheric CO_2 in units of ppmv per year (1 ppmv per year equals 2.12×10^9 tons C per year) and a measure of the gradient in CO_2 between the northern and southern hemispheres (_) expressed as the difference between the concentrations of CO_2 measured at Mauna Loa (MLO) and South Pole (SPO) (ppmv).

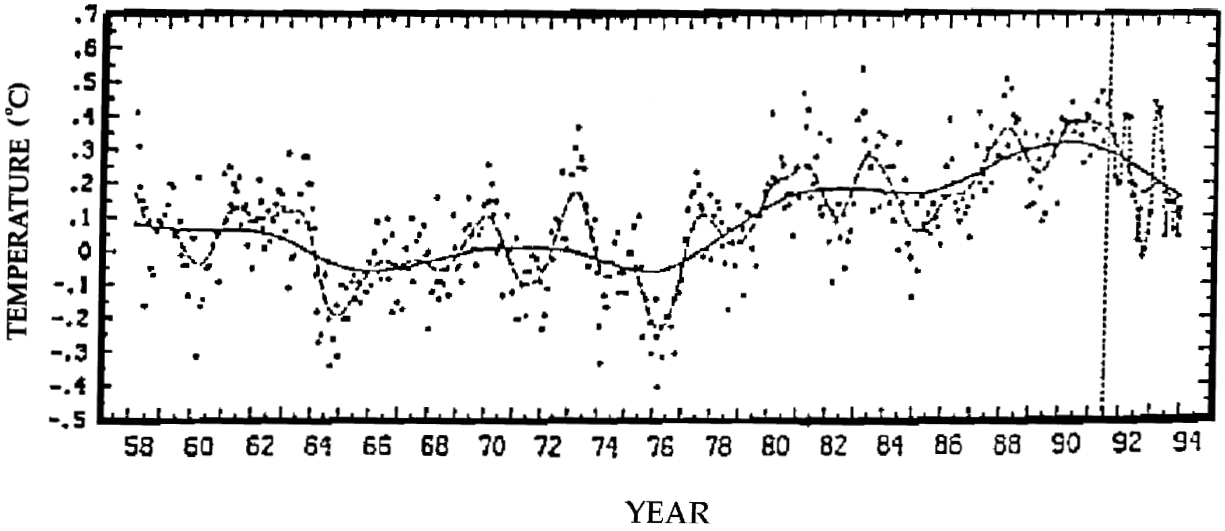

FIGURE 9 Globally averaged sea surface temperatures from 1958 to 1994. Timing of the eruption of Mount Pinatubo is indicated from the dotted vertical line (adapted from Keeling and others, 1995).

Summit in Rio de Janeiro in 1992. Anecdotal evidence suggests that the pace of tropical deforestation has increased more recently.

C.D. Keeling and co-workers[7] used a combination of CO_2 data and measurements of the isotopic composition of carbon in CO_2 to isolate influences of the ocean and the biosphere-soil system on the trends in CO_2 observed since 1978. Between 1980 and 1989, they found that the concentration of CO_2 rose more rapidly than expected, given the earlier trend (that is, if fossil fuel had continued as the major source, we would have needed to assume a higher airborne fraction to account for the data in the 1980s). The anomalous rise ended in 1989; by 1994, concentrations of CO_2 had reverted to the long-term behavior observed before 1980. As illustrated in figure 9, adapted from C.D. Keeling and co-workers,[7] globally averaged surface temperatures rose steadily over the decade of the 1980s, peaking in 1990 and declining by several tenths of a degree after the 1991 eruption of the volcano on Mount Pinatubo. They concluded that, with reference to the long-term trend, there was a net cumulative increase of about 2.3×10^9 tons of carbon released from soils and the biosphere from 1980 to 1989 with a compensating increase of 3.5×10^9 tons in carbon taken up by soils and the biosphere from 1989 to 1991. Those numbers, when expressed in terms of mean annual-exchange rates, are small relative to the mean annual uptake of 2×10^9 tons of carbon per year inferred for the 1990s by R.F. Keeling and co-workers[4]: Averaged over the period 1980–1991, the anomalous biosphere-soil uptake inferred from the isotopic analysis amounts to less than 10^8 tons of carbon per year. The pattern reported by C.D. Keeling and co-workers[7] is consistent generally with results based on the CO_2–O_2 analyses discussed above. They argued that higher ocean temperatures contributed to a cumulative reduction of 2.9×10^9 tons in carbon uptake by the ocean over the period 1980–1991; again, this is small relative to the annual mean uptake of 1.7×10^9 tons of carbon per year inferred from the CO_2–O_2 analysis. There are additional indications in the isotopic data of an influence of El Niño. Release of carbon from the tropical ocean is reduced as a consequence of suppressed upwelling during El Niño. At the same time, it appears that tropical terrestrial environments are responsible for increased release of CO_2 associated most probably with regional changes in tropical climate.

Observations of turbulent exchange of CO_2 between the atmosphere and a deciduous forest in New England (Harvard Forest in central Massachusetts) provide independent support for the existence of a middle-latitude Northern Hemisphere sink for CO_2.[8] Net ecosystem exchange of carbon varied from a low of –1.4 tons per hectare

per year in 1992–1993 to a high of –2.8 tons per hectare per year in 1990–1991 (negative values indicate that the forest represented a net sink for atmospheric CO_2). Interannual variability was associated with changes in climatic conditions and was sensitive particularly to the length of the growing season (regulated primarily by temperatures in spring and early fall), cloud cover in summer, drought in summer, snow depth, and other factors that affect temperatures of soils in the dormant season. If we were to assume that Harvard Forest was representative of forests and woodlands in North America (obviously a leap of faith) and adopt an average of the range of values observed for carbon uptake at Harvard Forest over the period 1990–1995, we would conclude that forests and woodlands in North America could be responsible for net regional uptake as large as 1.3×10^9 tons of carbon per year, with comparable regional emissions of 1.5×10^9 tons of carbon per year in 1991 contributed by combustion of fossil fuel.

Additional evidence supporting a Northern Hemisphere sink for CO_2 comes from an analysis by Fan and co-workers[9] of measurements of CO_2 reported from a distributed network of 62 sampling stations.[10] Using 2 distinct general-circulation models for the atmosphere and a model to describe ecosystem exchange of carbon, they concluded that the combination of biosphere-soil systems in Eurasia and North America accounted for a net CO_2 sink of about 2×10^9 tons of carbon per year for the period 1981–1987, with somewhat larger removal in 1988–1992. The sink inferred in their analysis is remarkably similar in magnitude to the result derived above. Their study suggested that the sink is primarily in temperate regions of North America and in the boreal forests of northern Eurasia. Consistently with the speculation above, they raised the possibility that release of CO_2 associated with combustion of fossil fuel and cement manufacture in North America can be offset significantly at present by uptake in temperate forests.

I have shown that geographically distributed measurements of O_2 and CO_2, in combination with data on the isotopic composition of CO_2, can be used to place important constraints on the budget of atmospheric CO_2. A convincing body of evidence suggests the presence of an important and persistent sink for CO_2 associated with the biosphere-soil system in the Northern Hemisphere. The analysis indicates that the sink can accommodate as much as one-third of the carbon added to the atmosphere today by the burning of fossil fuel. The sink is most likely in regions of North America and Europe where forests were once abundant but where overuse resulted in their depletion (early in Europe, more recently in North America). As coal replaced wood as a primary fuel, and as industrial practices evolved, forests were allowed to regrow. The sink for CO_2 at northern middle latitudes could be temporary and likely to diminish in importance as the biosphere-soil system approaches a new steady state reflecting mixed current patterns of land use.

IMPLICATIONS FOR THE FUTURE

Soils at high latitudes provide an important reservoir for organic carbon—between 200 billion and 500 billion tons of carbon.[11–13] The eddy-correlation method used to study exchange of carbon between the atmosphere and Harvard Forest has been applied also to the carbon balance of a mature black spruce forest in central Canada.[14] It was found that decomposition of organic carbon in soils of this system resulted in a small but important net source of CO_2 emission to the atmosphere—0.4 ton of carbon per hectare per year—in1994–1996. Emission rates increased by a factor of 10, however, as temperatures rose from –2° C to 5° C, raising the possibility that high-latitude soils could be a much more important source of CO_2 in the future if the climate warms significantly at high latitudes.

In forecasting upcoming trends in CO_2, it will be important to allow for feedbacks between the climate system and the complex suite of processes that regulate the distribution of carbon over its dominant reservoirs—the atmosphere, soils, biosphere, and ocean. As indicated by the analysis of C.D. Keeling and co-workers,[7] a warmer ocean might constitute a less-efficient sink for excess concentrations of atmospheric CO_2. It appears that the global biosphere-soil system played a relatively minor role in the budget of CO_2 over the last 40 yr, with release from tropical ecosystems offset by regrowth of forests at northern middle latitudes. But the signature of the biosphere-soil system could change over the next few decades as middle-latitude forests approach a new steady state, completing their recovery from earlier disturbance. The global biosphere-soil system could switch from its current neutral role to become an important net source of CO_2 if higher temperatures develop, stimulating an increase in emissions from soils at high latitudes.

The analysis indicates that for most of the last 40 yr, tropical ecosystems might have been a net source of CO_2 for the atmosphere, equal to as much as one-third of emissions associated with global combustion of fossil fuel; that is, the excess of CO_2 released by deforestation in recent years over CO_2 absorbed by plants in regions deforested earlier but abandoned might amount to close to 2 billion tons of carbon per year. This suggests that the climate-change issue is linked inextricably to the challenge of preserving the rain forests. Such countries as Brazil and Indonesia could bear responsibility as much as the United States and China for the contemporary buildup of atmospheric CO_2.

The suggestion that regrowth of forests in North America can provide a sink for CO_2 comparable with that associated with local consumption of fossil fuel, although plausible, is clearly speculative, on the basis of current evidence. It merits further attention, however. If validated, it would complicate the task of those charged with developing a strategy to minimize the future growth of greenhouse gases in the atmosphere.

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Discussant

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I will comment on some of the remarks of Michael McElroy from the viewpoint of a climate modeler. Paleoclimate data are limited in space and limited in the variables that they can reveal, but they pose an important challenge to models because they represent climates different from today's, against which our models are necessarily calibrated. Paleoclimate data give us a unique opportunity to see the excursions that climate can undergo. It is a major challenge to models to be able to recreate selected paleoclimates and someday to simulate the changes from 1 paleoclimate to another.

Nature follows only 1 path, but models can be run under many conditions; this is a major advantage of modeling. We can change the internal structure of a model, we can change the external forcing, and we can run large numbers of climate simulations. Thus, we have been able to simulate with reasonable confidence the climate of an ice age, for example, but the transition between an ice age and the intervening interglacial periods has not yet been stimulated. As valuable as paleoclimate data are, they represent only a slice-of-time "validation of opportunity" as a supplement to model validation with the 4-dimensional instrumental data on the current climate.

Michael McElroy also referred to the ocean-atmosphere system. We have had climate models of one sort or another for some 40 yr. These have been developed by the atmospheric and oceanic research communities more or less independently. The workhorse of modern climate modeling is the coupled model in which atmosphere, ocean, cryosphere, and the land surface are joined to form an interacting dynamic system.

Some 20 groups now have coupled models. Many have performed simulations covering a century or more in which solar radiation and atmospheric composition are given modern values. These simulations show irregular fluctuations on seasonal, yearly, and decadal time scales when there are no changes in external conditions. We refer to these fluctuations as natural climate variability—fluctuations that occur without a change in the forcing and that are essentially unpredictable. In long-term integrations, these fluctuations tend to average out, but they are partly responsible for the systematic errors found in every model. On the whole, however, current climate models do a reasonably good job of simulating the average large-scale seasonal distribution of climate.

One important application of coupled models in recent years has been in simulating the climatic consequences of increased CO_2 concentration in the atmosphere. In general, such experiments yield a warming of several degrees Celsius, with larger warming over the continents than over the oceans. When we search the observed record of the last few decades, during which CO_2 in the atmosphere has increased by perhaps 30%, we find almost no correspondence between the models' simulations of warming for this CO_2 increase. This has led some to conclude that the models are wrong. But probably the models are simulating the nearly correct response to CO_2 increases, and other forces are operating in nature.

More recent simulations with coupled models consider the transient increase in both CO_2 sulfate aerosols, which have a cooling effect that might offset some of the CO_2-induced warming. When such runs are made for 100 yr or so (it takes about 70 yr to double the CO_2 concentration), we find that the large-scale pattern of surface-air-temperature changes simulated by the models bears a marked resemblance to that observed over comparable periods.

That important result was highlighted in the 1995 report of the United Nations Intergovernmental Panel on Climate Change, which contains the now-famous statement that "the balance of evidence suggests a discernible human influence on global climate." This finding has now been verified by several models in the continuing search for the details of an anthropogenic climate-change "fingerprint."

We cannot, however, predict all aspects of a future climate. Whether a warmed climate, modified by aerosols, will increase the frequency of El Niño or change the intensity or onset of monsoons, for example, has not yet been established. Coupled models, like their atmospheric and oceanic components, contain systematic errors that need to be reduced. As we add interactive biology and chemistry to the models, they will become even more complex, but presumably they will also be more accurate.

The climate-modeling community spends a great deal of effort in building models and in deciding on the parameters for individual physical processes, such as cloud formation, radiation, and ocean mixing. We also use a great deal of computer time in integrating the models over hundreds or even thousands of years. But we pay relatively little attention to the systematic extraction of information from the results. In some ways, climate modeling is in a situation analogous to that of biology, which (as we have heard here) is trying to move from the genome scale to the organism scale. We know something about the individual components of climate models, but we are not very clever in diagnosing the behavior of the coupled climate system—we cannot point to an error as having specific causes. Such model diagnosis and interpretation of model performance is an underdeveloped part of climate research.

Discussant

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The Department of Energy (DOE) has been a pioneer in the use of computers. As we go into the next generation of climate models, we will be trying to address several major problems. One, of course, is to increase the spatial resolution of the models. This is very important for understanding the atmospheric circulation that is generated by the models and the ocean and sea-ice components of the models. We are finding, for example, in the ocean models that at very high resolution the models generate realist current systems that can be validated against observed satellite and in situ data. However, there are still some substantial biases, and the oceanographic research community is trying to address them. With the advent of highly parallel and cluster computer systems that we see becoming available in the next few years, we should be able to exploit such computers in going to the next generation of climate models.

One persistent problem in climate modeling has been to identify the physical constituents that are not working properly. Clouds have always been very high on the list. I am pleased to see that, under the ARM program, DOE has taken the leadership role in trying to identify the defects of the models with respect to radiation processes.

Another issue in climate modeling is how we handle atmospheric water in all forms—water vapor, liquid, and ice. We are making substantial inroads in this regard in our models.

The chemistry of the carbon cycle, the sulfur cycle, and other cycles in the atmosphere and in the oceans is important. Instead of just having models with specified concentrations of these gases and aerosols, we will have models that can predict changes in chemistry and in aerosol distributions.

Modeling of ecology is also important. We are moving to models with realistic ecologic systems that take into account the vegetation of the tropics, prairie, middle latitudes, and tundra in a much more accurate manner. The different systems will be validated with satellite data and other field data.

To make full use of those models, we need to look at the interface between the climate-model simulations and societal and economic variables more closely. This field is called integrated assessment. DOE has been a leader in trying to explain how to use interaction of economic models with climate models to produce information useful to policy-makers and the public.

Now that we understand most aspects of the climate system, including the effects of burning fossil fuels and other climatic forcings, we should not just have a simple view. This is not the time to slack off in funding global-change research. I think, with the wide range of uncertainties and probably some surprises in our understanding, that this is not the time to decrease support for a very aggressive research program.

Discussant

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I would like to express my gratitude to the Department of Energy for its long-term support of multidisciplinary research on the environment. It is the only federal agency that has made such commitment, which I hope will continue.

My comments pertain to the role of the oceanic biota and climate feedback. By *feedback*, I am referring to what climate does to affect the abundance and distribution of organisms and to what organisms do to affect biogeochemical cycles that, in turn, influence climate. Understanding such feedback is critical not only for evaluating how natural climatic changes that are recorded in the geologic history of the earth have been affected by and affect biologic processes, but also for using historical data and observations to represent such processes in general-circulation models that are used to predict climatic change and its potential effects. The researchers who have developed general-circulation models are trained primarily in physics. The language of biology differs from that of physics, so communication between them is often strained. I will briefly illustrate how critical it is to bridge the language barrier in climate-change research and thus how essential a multidisciplinary approach is for developing a comprehensive earth-system science program. The latter is a great challenge in the coming decade.

A century or so ago, scientific disciplines were not so segregated from each other, and individuals could be truly multidisciplinary researchers. In 1830, the British geologist Charles Lyell[1] published the first volume of his classic text, *Principles of Geology,** in which rational hypotheses were developed to explain geologic formations. Because geologists relied heavily on fossil biologic groups to identify epochs, Lyell was a superb biologic taxonomist and was able to identify numerous fossil marine invertebrates, many of which appeared to be extinct. While on a trip to the continent, he noted that marble columns of the Roman ruins of the Temple of Scerapi in Pozzuoli (just south of Naples) had obvious signs of marine boring organisms. The columns, which were (and still are) above water, must have been partially submerged in the ocean for some period after the construction of the temple but before Lyell's visit to it. How could the temple have been under water, and how did it come back to the surface?† Similarly, why were there fossils of marine origin high up in the Alps and Dolomites? Were the mountains under water at some point as well? Moreover, why were many of the fossil organisms extinct?

It is difficult to imagine today what explanations had been offered for those phenomena before Lyell. They included eruptions of submarine volcanoes that spewed marine organisms onto the land, evidence of the great flood and later subsidence of waters as described in Genesis, and clear evidence that God was testing one's belief (the "fossils" were not really extinct marine organisms, but rocks made to look like marine organisms to fool one into believing in false creations). Lyell elaborated on the absurdity of many of the hypotheses and went on to attribute the changes in the earth's surface to causes still in operation. Thus, earthquakes, floods, volcanism, and erosion (causes now in operation), shaped the earth. The presence of fossils of marine organisms in the Alps must mean that the mountains (or the rocks of which they are made) were at one time under water. Lyell's concept, which came to be known as *uniformitarianism*, forms a fundamental paradigm in geology and geochemistry to this day‡ and is embraced (mostly unconsciously) by climate-change researchers. It is used, for example, to explain

*The text was published just before Darwin's departure on the *Beagle* in December 1830, and Darwin took a copy with him. Darwin was extremely impressed and influenced by Lyell, and many of Darwin's early studies focused on biogeologic processes, such as how coral reefs are formed and maintained. Darwin was elected to the Royal Society in 1838 for his contributions in geology; his fundamental concepts of evolution were not published until 1858.

†The temple was built on a highly elastic plate that undergoes periodic uplifting and subsidence in response to subsurface volcanic pressure fields. The apparent changes in sea level can be a meter or so per century in the region.

‡Originally, Lyell's concept of uniformitarianism stressed that the processes responsible for changes in geological structures were a result of processes that had occurred at the same rate over geologic time. That notion has been replaced by the concept of punctuated events, or series of events, that shape the earth's surface. Nonetheless, the original concept catalyzed a rigorous systematic analysis of geologic formations that forms the basis of contemporary geologic theories.

glacial and interglacial (or paleoclimatic) changes on the basis of astronomically predictable variations in solar-energy fluxes (such as "Milankovich cycles"), meteorite impacts, volcanism, changes in ocean circulation and heat transport, and so on. It is also used to rationalize changes in the radiative forcing resulting from variations in greenhouse gases, such as CO_2, which in the past were generally believed to have been a response to, rather than a cause of, climate change.

An ecologic analogue of uniformitarianism was succinctly stated by G. Evelyn Hutchinson, who wrote that "the ecological theatre of the present is only a scene on the ongoing evolutionary drama." This statement implies that ecologic processes must be considered within a continuum of change, not the least of which is a consequence of climatic variation. In the roulette wheel of evolution, some organisms have persisted beyond and despite large changes in climate, and others have become extinct or have evolved by natural selection to adapt to new conditions. Among the most-persistent and most-dynamic organisms (from an evolutionary perspective) are marine unicellular algae, the phytoplankton. (*Plankton* is from a Greek word meaning "wandering"; phytoplankton are photosynthetic organisms that drift with the currents.)

Let us now turn to the ecologic theater of the contemporary interglacial ocean. Satellite imaging has provided high-resolution pictures of the chlorophyll distributions in the oceans. High chlorophyll (that is, phytoplankton) concentrations are found in coastal regions and in the open ocean, where physical processes provide a source of nutrients, light, and simultaneous stability within the water column. Low-chlorophyll regions, characteristic of most of the central gyres (circular ocean currents), is also a consequence primarily of physics; in such regions, the vertical flux of nutrients from the ocean interior is relatively weak. The phytoplankton in the oceans are extraordinarily diverse from a genetic viewpoint, belonging to over 18 phyla (all flowering plants on land belong to a single phylum), and, although their composite biomass accounts for only about 10^{12} kg of carbon, they fix about 40×10^{12} kg of CO_2 per year. For comparison, terrestrial plants, which account for about 500×10^{12} kg of carbon biomass, fix about 60×10^{12} kg of carbon per year. Hence, marine phytoplankton are more than 300 times as productive per unit of biomass as their terrestrial counterparts. The productivity of marine phytoplankton is limited primarily by the availability of essential nutrients, such as nitrate and phosphate, but not CO_2.

In the ocean, about 15% of the phytoplankton organic carbon sinks out of the upper ocean into the interior, where it is oxidized back to inorganic carbon. In so doing, marine phytoplankton help to maintain a negative diffusive gradient between the ocean and atmosphere that drives a nonequilibrium flux of carbon between the 2 reservoirs. This process, called the "biologic pump," is analogous to the decomposition and burial of organic matter (such as leaf litter) in terrestrial ecosystems. The oceans differ from terrestrial systems, however, in that the carbon stored in the oceans resulting from biologic activity is primarily inorganic carbon. Only a very small fraction of the organic carbon is preserved in the sediments. Over geologic time, a small fraction of the organic carbon preserved in the sediments undergoes chemical transformations that lead to the production of oil. Simply put, oil is relic fossil phytoplankton. The burial of organic carbon results in the net release of oxygen. Hence, the oxygen in the earth's atmosphere is fossil oxygen and reflects, in part, the burial and sequestration of fossil phytoplankton carbon in the oceans.

For carbon to be buried and oxygen evolved, phytoplankton production must not have been in equilibrium with oxidation processes; that is, there must have been a change in the biologic pump on geologic time scales. How can such changes occur, and what is their effect on atmospheric CO_2? Geochemically, the most-obvious mechanism for enhancing the biologic pump is to add nutrients to the ocean. But which nutrients, and how should they be added? Let me briefly consider a particular nutrient and its consequences.

In the eastern equatorial Pacific, there are high concentrations of the essential plant nutrients nitrate and phosphate in the upper ocean, but phytoplankton chlorophyll concentrations are remarkably low. In a series of experiments in the 1990s, infinitesimal concentrations of iron were added directly to a small region of the high-nutrient, low-chlorophyll waters. The iron additions led to dramatic and immediate increases in phytoplankton biomass and photosynthetic fixation of carbon. In fact, photosynthetic rates more than doubled within 24 hr, and high rates were sustained for several days, until the iron was depleted. It turns out that a major source of iron for the oceans is aerosol deposition, particularly wind-driven transport of dust from deserts. Desertification leads to enhanced production of wind-blown iron, and analyses of ice cores suggest that the flux of such iron was about 100

times as high during glacial periods as it is today. Perhaps this flux stimulated the biologic pump and contributed to the drawdown of atmospheric CO_2 during glacial periods.

Iron is extraordinarily important in the global nitrogen cycle. With very few exceptions, phytoplankton must use a fixed-nitrogen source for growth. Nitrogen fixation is carried out by a very small number of species of cyanobacteria, whose abundance in the modern ocean appears to be extremely low. The distribution of nitrogen fixers appears to be highly correlated with the flux of wind-blown iron. A small change in the amount of nitrogen fixed could have a large effect on the net photosynthetic fixation of CO_2. [2]

Thus, iron can both directly affect biologic fixation of CO_2 in the high-nutrient, low-chlorophyll regions of the oceans and indirectly affect the same process in the other regions by limiting nitrogen fixation. Will the flux of iron to the oceans change in the coming decades? If so, how, and in what direction, and how will the change affect the biologic pump? My point here is not the specific process of wind transport of iron or its effects, but the lack of recognition of the process in coupled atmosphere-ocean general-circulation models. Virtually all modelers assume that oceanic biology is in a steady state and that the net uptake of atmospheric CO_2 by the oceans is accomplished by physical-chemical processes without any biologic enhancement. That is certainly not true on geologic time scales, and it is unlikely in the coming decades. If such feedback processes as I have described are ignored, model prognostications with regard to atmospheric CO_2 and its effects will be quantitatively, if not qualitatively, incorrect.

Many people doubt that human influences on CO_2 have had any effect on the earth's climate. Without a control in the experiment, it is extremely difficult to prove beyond doubt that changes in climate since the Industrial Revolution are a direct consequence of human activities. As Michael McElroy stated, ice-core data clearly indicate that current atmospheric concentration of CO_2 are about 30% higher than at any time in the last several hundred thousand years. We do not completely understand climate-change processes in the geologic past, but if the consensus interpretation of the climatic forcings and feedbacks during the Holocene is even roughly correct (I assume that the consensus interpretation of the glacial-interglacial forcing is based on Milankovich cycles, with a feedback on ocean chemistry and biology that led to changes in atmospheric CO_2), such information cannot necessarily be a guide to the present situation, in which changes in atmospheric gas composition are assumed to be a cause, rather than a consequence, of climate change. Nonetheless, as I have briefly described, many complicating and interactive biogeochemical processes can attenuate or amplify the direct effect of anthropogenic emissions of CO_2. Unless the ecologic feedbacks are included with the physical processes in mathematical representations of the earth's climate system, coupled atmosphere-ocean models will remain crude approximations of a complex system.

In conclusion, I would like to return to a quotation from Charles Lyell[1]: "The system of scholastic disputations encouraged in the Universities of the middle ages had unfortunately trained men to habits of indefinite argumentation, and they often preferred absurd and extravagant propositions, because great skill was required to maintain them; the end and object of such intellectual combats being victory and not the truth." The debate on the causes and consequences of change in the earth's climate system should be vigorous and open and should include of biologic feedbacks. As scientists, we have an obligation to search for the truth. As human beings, with families and children, we should recognize that the potential consequences of our research on our lives and quality of life could be profound. The search for the truth will not be simple; it will require substantial vision and consistent commitment on the part of the Department of Energy and other federal agencies to long-term multidisciplinary research.

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Restoring the Environment Via Bioremediation and Molecular Sciences: Prospects for Better Understanding and New Science-Based Solutions

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There is a tremendous national and international legacy of environmental contamination—from defense production, general industrial activity, and agriculture—that poses an enduring threat to the health of humanity and to the survival of ecosystems throughout the biosphere. The bulk of this contamination occurred in a relatively short time between the Second World War and the time in the early 1970s when the long-term adverse effects of such contamination were first realized. Stringent regulations have halted further release of such materials, but the problem of restoring the environment and protecting the health of future generations largely remains, and is proving to be a daunting challenge. While the problem of extracting or passivating contaminants in the environment has proven to be vastly more complex than first imagined, an intractable system of regulations and practices has made it even more difficult to bring the benefits of sound scientific research to actual cleanup efforts. The conservatism built into the system has led to environmental restoration's focus on proven, albeit dated, science and simple technologies. Associated bureaucracies related to the legal requirements have been proven to be ineffective and costly.

However, this is a time of unprecedented progress in scientific understanding of phenomena in the environment, and evolving capabilities in empirical and theoretical research promise a new era in scientific advances, from the molecular to the system level. In addition, our understanding of the risks posed by substances in the environment is evolving rapidly. It is critical at this time to capitalize on these opportunities.

Progress has occurred on numerous fronts. There has been tremendous progress in our understanding of ecosystems in general, and in the ecosystems of subsurface environments especially. The discovery of the existence of extensive deep subsurface microbiology , its complexity and its functionality, is revolutionary. There is an unexpected diversity in microbial communities: Microorganisms and organisms in other phyla exhibiting the ability to express selected genes under stress, and genetic sharing of traits between members of a microbial community (consortium) and between species. Many of the traits exhibited by subsurface communities are common to the traits exhibited by drug resistant microbial communities now bedeviling the health profession. A picture of a robust and complex response by microbial communities (consortia) is emerging, making bioremediation feasible. Whereas genetic engineering was only recently deemed necessary to enable microbes to deal with

pollutants that arise from human activities, the genetic diversity and complex functionality of native communities now suggest that natural attenuation may be the basis for long term solutions, in many cases obviating intervention. Monitoring and modeling replacing active intervention.

Modern tools from the physical sciences, computing sciences, and life sciences promise explosive growth in knowledge in both the predictive and the empirical development of these problems. Computational sciences have shown an exponential growth with the advent of massively parallel systems and are poised to carry us past the reductionist paradigm for science. Computational science will soon be capable of dealing with the systems-level complexities of environmental contingencies. Likewise, the emerging generation of experimental tools that use advanced physical probes—such as synchrotrons, nuclear magnetic resonance, laser-based tools, and surface science—brings revolutionary sensitivity and analytical richness to practical systems studies. And genomics, structural biology, and molecular-level mechanistics are becoming the tools of choice for the study of all advanced biological systems.

An improved mechanistic understanding of health effects and the robustness of genetic defense and repair processes are changing our idea of the ultimate impact of environmental exposures to broad classes of compounds and elements. New classes of persistent manmade chemicals in the environment, e.g. endocrine disruptors, are joining genotoxins and other carcinogens as critical targets for research and eventually for control and mitigation.

Possibly most important, the information revolution promises to cut across historical institutional, and disciplinary boundaries to bring concepts and capabilities together in new ways, focused on problem solving, which will transform the research paradigm. Tools are emerging to bring teams of people and experimental and computational capabilities together in a real time, vertically integrated environment. It is an exciting time for conducting research that can contribute to science-based solutions to the Department of Energy's (DOE) environmental restoration challenges, and the DOE Biological and Environmental Research (BER) program is well-positioned to make significant contributions to those solutions.

THE NATIONAL WASTE LEGACY

To appreciate the areas in which further scientific advances are needed, the magnitude of contamination must be considered. Across the country there are more than 20,000 declared hazardous material generators. Hazardous waste is routinely sent to more than 5,000 waste treatment, storage, and disposal facilities. Inventories have listed more than 600,000 leaking underground-storage tanks in the nation. As previous hazardous waste sites are characterized, there could be as many as 32,000 national CERCLA sites.[1]

Furthermore, the nation has responsibility for approximately 6,000 contaminated federal facilities.[2] For example, DOE is responsible for waste management and environmental restoration of more than 130 installations across the United States. Contaminated media at DOE's facilities include about 1.85×10^9 m^3 of water (99% groundwater), and 79×10^6 m^3 of solid media (95% soils and sediments).[2] About 56% of the waters show radiologic contamination only, 30% mixed hazardous and radiologic contamination, and 14% hazardous contamination. Radiologic contamination of soils exceeds 70% with equal proportions of mixed and strictly hazardous contamination.[2] Many of the contaminants are widely distributed in groundwater and in soil and sediments at low concentrations across many diverse geologic settings.

A broad variety of contaminant classes were disposed at DOE's facilities.[3] The most-common classes were fuel hydrocarbons, chlorinated hydrocarbons, radionuclides, metals, and ketones; the most-common substances were toluene, trichloroethylene, tritium-uranium, lead-chromium, and acetone. More than 50% of DOE's waste sites were contaminated by binary or ternary mixtures, with the most-common binary-contaminant mixture consisting of metals and radionuclides.[3] Twelve other common pairings included metals, anions, radionuclides, chlorinated hydrocarbons, polychlorinated biphenyls (PCBs), and ketones in various combinations.

Many of the combinations resulted in mixtures of contaminants that could interact with each other to modify contaminant subsurface geochemical behavior. Mixtures of radionuclides and metals with organic ligands (organic acids or amino-carboxylic chelating agents) can form mobile aqueous complexes in soil and groundwater. Likewise, organic solvents (chlorinated hydrocarbons and ketones), which can mobilize sparingly soluble hydrophobic organic compounds, were disposed of with PCBs. Organic compounds that can stimulate subsurface microbial

activity, thereby modifying speciation of metals and radionuclides, were disposed of simultaneously with metal- and radionuclide-containing wastes.

The volume and extent of contamination of diverse subsurface environments creates a pressing need for effective remedial technology. Early remedial approaches, such as excavation and groundwater pumping and treatment, have proved inadequate to address the problem.[4] Attention is now focused on insitu technologies that include bioremediation, intrinsic contaminant degradation, and contaminant containment and treatment with insitu permeable chemical barriers.[5] There are several advantages to these approaches: Because they are insitu technologies, the wastes are contained and treated on site, contaminants can be completely transformed to nonhazardous materials, and the public perception is one of "natural processes." These technologies also have disadvantages: Because they are insitu technologies, the methods must be adapted to the specific chemical, physical, and biologic conditions of each site; monitoring and assessment of successful contaminant transformation are difficult, and the concentrations of process end products can exceed regulatory requirements. All these approaches require a detailed understanding of the physical, chemical, and biologic processes that occur in subsurface environments.

The DOE BER program has had a long-term commitment to basic research that provides the scientific basis for environmental-restoration technologies. The success of any of these approaches depends on coupling process-level understanding with long-term monitoring of contaminated systems that are undergoing treatment to pre-established end points. In the case of insitu bioremediation, successful remediation requires knowledge of the diversity and function of indigenous microbial communities, of the expression of degradative genes that might be present in the population, and the chemical reactivity of contaminants. To assess the success of intrinsic contaminant degradation, additional information is needed on the nature of solute and microbial transport in subsurface environments, which in turn requires knowledge of subsurface physical and chemical heterogeneity. Traditional sampling, measurement, and monitoring strategies for subsurface environments are expensive and time-consuming, and they provide a complete view of the range of microbial ecology in the subsurface. When insitu permeable chemical barriers are considered for use at contaminated sites, similar knowledge on the subsurface environments is needed. The gaps in understanding of how complex hydrologic, geochemical, and biologic processes interact to affect subsurface contamination have formed the basis for much of the BER program's research aimed at restoration of DOE lands.

BER PROGRAM ACCOMPLISHMENTS

The BER program's studies in subsurface science since 1985 have contributed substantially to the fundamental understanding of microbial ecosystems in pristine aquifers and unsaturated zones. Over the last decade, BER has sponsored research in deep microbiology and bacterial transport at multiple DOE and private field sites and was responsible for developing techniques to collect samples that could be unequivocally demonstrated to host microorganisms representative of indigenous microbial ecosystems.[6–9] Diverse bacterial communities were detected in a range of geologic environments not previously known for hosting microbial communities, including coastal-plain sediments in eastern Georgia, consolidated rock almost 3 km deep in sedimentary basins in Virginia, and basaltic aquifers in central Washington.[6,10] Researchers have used 16s ribosomal RNA (rRNA) probes to determine the phylogeny of many of the ecosystems and to relate microbial strains isolated from the subsurface to known genera.[11,12] In many cases, the large numbers of the organisms within subsurface samples could not be identified; this showed the uniqueness and potential long-term isolation of these environments. Some of these communities produced novel and unique organisms with previously unsuspected capabilities to degrade anthropogenic compounds.[13,14] A new chemoheterotrophic strain of *Sphingomonas, Sphingomonas aromaticivorans*, was identified as part of the consortium present at the DOE's Savannah River site. The species catabolically degrades a broad array of aromatic compounds, including xylene, toluene, and naphthalene; the ability to degrade these compounds was determined to be encoded on a 180-kb plasmid (figure 1).[13,15,16] These findings highlight the ability of the subsurface community to degrade and transform particular contaminants.

Many of the organisms have adapted to deep subsurface environments, as indicated by the uniqueness of their metabolism. The BER program supported the discovery of a bacillus, *Bacillus infernus*, that is a strict anaerobe (it cannot tolerate oxygen).[10] The *B. infernus* strain was isolated from rock fragments collected at depths of 2.65–

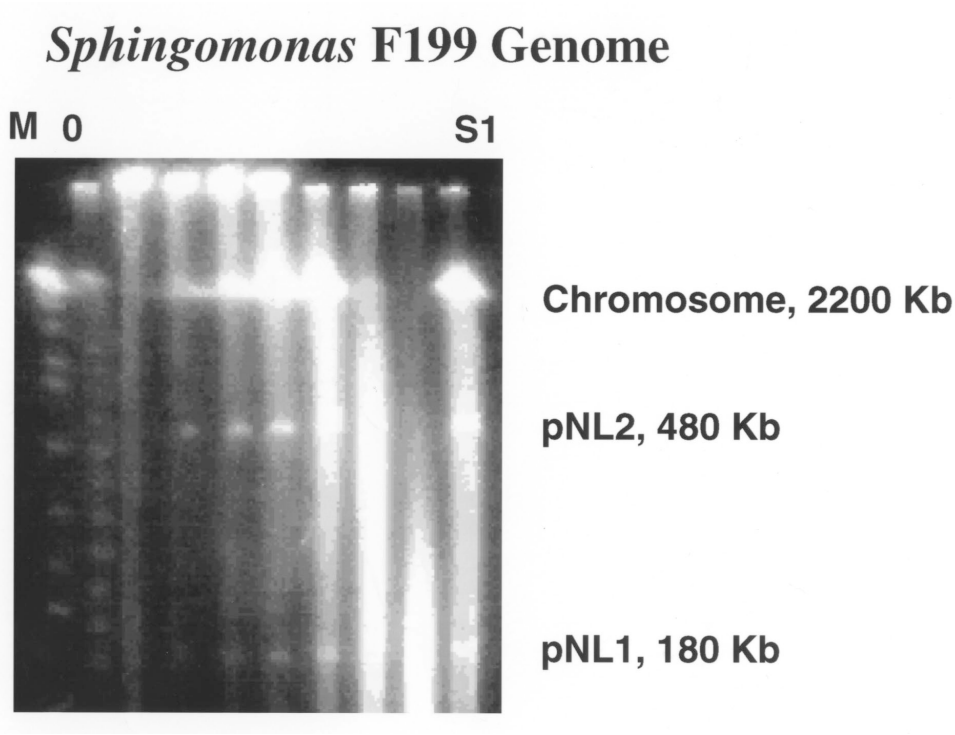

FIGURE 1 Gel blot of F199 genomic DNA showing presence of 2 megaplasmids, including pNL-1 at 180 kb, which appears to encode for degradation of aromatic compounds (Kim and others, 1996).

2.77 km in the Taylorsville Basin of Virginia. The bacterium can grow by fermentation of glucose or oxidation of formate or lactate accompanied by the reduction of Mn(IV) and Fe(III). Elsewhere, program-sponsored research in the biogeochemistry of subsurface systems used laboratory studies to examine the mechanisms of dissimilatory iron reduction, which has been inferred to be responsible for the close spatial association of subsurface microorganisms and secondary iron-bearing mineral oxides, such as goethite.[17] Experiments demonstrate that such iron oxides can play an important role in the chemical transformation of contaminant complexes.[18,19] Some of the concepts of long-lived, subsurface permeable barriers call upon microbially catalyzed iron reduction as an integral part of the remedial treatment of metals and radionuclides.[5] The program's research findings indicate that microbial populations with metabolism adapted to perform the kind of subsurface metal oxidation and reduction important to contaminant transformation are already present in the subsurface.

Previous research addressed the issue of how microbial ecosystems were established in subsurface environments. Questions about the availability of sufficient nutrients and energy sources and the rates of nutrient and bacterial transport were addressed in transport studies conducted in intermediate-flow cells and in field settings. In intermediate-scale experiments in the laboratory, researchers have studied the transport and distribution of bacteria in physically and chemically heterogeneous porous media specifically constructed to determine the complex interactions between the heterogeneities.[20] Microorganisms have been discovered to position themselves at interfaces of physically heterogeneous media because of the chemical heterogeneities in electron-acceptor concentrations that arise at such interfaces. In field studies, the transport of bacteria through aquifer systems often appears to be limited by the physical constraints of the porous media; results from intermediate-flow cell experiments allow scientists to evaluate the magnitude of the contribution made by solute flow to the residence time and availability of nutrients and electron acceptors.[20, 21]

The role of microbial ecosystems in buffering the aqueous geochemistry of aquifers has been explored by BER program researchers using stable-carbon isotopic geochemistry; as a result, the existence of anaerobic

subsurface lithoautotrophic microbial ecosystems that derive energy from geochemically derived hydrogen has been suggested.[22] Such ecosystems might constitute a potential model of how life can occur on other terrestrial-type planets, such as Mars. Just recently, the National Aeronautics and Space Administration's exobiology program has been willing to fund exploratory research in this field to explore how far through the solar system microbial communities extend.

The BER program's studies on the microbial genome were influential in providing the definitive genetic sequence to confirm the Woese rRNA determination of the Archaea as a separate major domain, with Bacteria and Eukarya, on the tree of life.[23] The determination of the complete genetic sequence for the extremophilic *Methanococcus jannaschii* revealed a high proportion of previously unknown genes and confirmed the uniqueness of Archaea relative to bacteria. The discovery allows re-examination of the question of the origin of life and assists in confirming the physicochemical conditions under which life first began.[24] Extremophilic organisms like *M. jannaschii* are being intently studied as possible sources of the enzymes important to new industrial processes, and knowledge gained about their natural enzyme systems can support the re-engineering of enzyme systems for specific functions, such as contaminant degradation.

FUTURE DIRECTIONS IN ENVIRONMENTAL RESEARCH

Much still is not known about subsurface microbial ecosystems, the degradative potential of microbial consortia, the mechanisms by which microorganisms degrade contaminants, and the effects of the microorganisms on insitu subsurface chemical and physical processes. A major need exists for an integrative and predictive capability that will allow coupling of molecular-scale mechanistic understanding with processes occurring on larger physical scales. The colonization of subsurface environments by microbial consortia is not yet fully understood. Transfer of genetic material among subsurface species is not well understood. There is also a need to develop the ability to design enzymes that can withstand extreme environmental conditions. The relationships between subsurface microbial ecosystems and macroscopic surface ecosystems have not been conceptualized from the perspective of complexity theory and self-organizing systems.

To address these kinds of research questions, the BER program has initiated another program in Natural and Accelerated Bioremediation Research (NABIR). The NABIR program builds on the BER program's decade of basic research contributions in subsurface microbial ecology and biogeochemistry and is aimed at providing the fundamental research to explain and manipulate the chemical, biologic, and physical processes that contribute to insitu bioremediation. The degradation or transformation of contaminants by microorganisms is seen as having great potential for meeting DOE's restoration challenges.

Modern physical and chemical tools are now available to address some of the unanswered questions about subsurface microbial ecosystems and demonstrate how more scientific knowledge will link to improved remedial approaches. Research sponsored by the NABIR program will use many of these tools.

Using the highly focused x radiation generated at new, third-generation light sources, such as the Advanced Photon Source at Argonne National Laboratory, and zone-plate collimation, scientists can explore the distribution of trace metals in complex microbial ecosystems associated with subsurface environments, such as plant-root systems.[25] The transformation of iron valence state as a function of depth within the crystal lattice can also be studied with synchrotron radiation, as evidenced by recent studies of dissimilatory iron-reducing bacteria at the Advanced Light Source at the Lawrence Berkeley National Laboratory.[26]

The BER program opened its first national scientific user facility, the William R. Wiley Environmental Molecular Sciences Laboratory at Pacific Northwest National Laboratory in Richland, Washington, in October 1997. The mission of the facility is to develop a molecular-level understanding of the physical, chemical, and biologic processes that underlie environmental remediation, water processing and storage, human-health effects, and atmospheric chemistry. The fundamental environmental molecular science that is conducted within the facility provides the knowledge base needed to address DOE's serious environmental issues, including the extensive contamination of soil and groundwater described earlier.

The complement of research equipment and general laboratory infrastructure within the Wiley Environmental Molecular Sciences Laboratory are grouped into three national user facilities: the High Field Magnetic Resonance

Facility, the High Field Mass Spectrometry Facility, and the Molecular Sciences Computing Facility. These facilities contain several one-of-a-kind and first-of-a-kind instruments that will support scientific advances in a variety of disciplines. For example, the High Field Magnetic Resonance Facility will contain one of the world's first 900-MHz nuclear magnetic resonance (NMR) spectrometers and a state-of-the-art 750-MHz NMR spectrometer. These instruments support studies of the molecular structure of enzymes, proteins, and DNA as related to bioremediation and cellular-response effects. The facility also supports a suite of modern pulsed electron paramagnetic resonance instruments that allow scientists to perform structural analyses of metal clusters important to enzymatic reduction of groundwater contaminants. Near-field optical microscopy permits the fluorescence imaging of single-chromophore probe molecules in specific ambient environments with nanometer spatial resolution. Detailed information on chemical reactions of individual molecules can be obtained through the combination of time-resolved spectroscopy and near-field optics. Environmentally important research includes the examination of membrane systems in plants, which can be extended by analogy to the membrane systems in microorganisms important to bioremediation.[27–29] A more-extensive discussion of the use of near-field microscopy for understanding biodegradation of chlorinated hydrocarbons is given by Dunning elsewhere in this volume. The Molecular Sciences Computing Facility contains one of the nation's fastest massively parallel computers, an IBM Scalable POWER Parallel System, which expands the capability to perform ab initio calculations of molecular structure for larger and larger single molecules. For example, in 1993 it was possible to perform an ab initio calculation of the structure of 18-crown-6 ether on a Cray supercomputer over a period of months; the computer in the new Molecular Sciences Computing Facility allows one to perform a similar computation on a Still's crown ether (a much larger molecule) within an hour or two. That example equates to an increase in computing capability by a factor of 100–1,000, which suggests that the nonlinearities and complexities of subsurface flow and transport can also be addressed more easily on such a machine. Dunning provides details of some of the research aimed at environmental restoration being addressed with the computational capability of this machine.

As the BER program initiates research in its NABIR program and brings the power of modern physics and biology to bear on key environmental processes related to bioremediation, the scientific community has the opportunity to contribute fundamental scientific research of direct relevance to addressing a major societal issue, the environmental legacy of the Cold War. The BER program's new national scientific user facility, the Environmental Molecular Sciences Laboratory is expected to provide resources to research teams nationwide for improving the understanding of molecular science related to the environment. It plans eventually to develop a field-research center for bioremediation that will allow questions of implementing bioremediation, including transport of microorganisms, to be addressed. These major opportunities for scientific advancement will be but the first of many sponsored by the BER program as it continues to foster basic scientific research into the next century.

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In the late 1980s, recognizing that the Department of Energy (DOE) would face enormous technical challenges in addressing its energy and environmental missions in the 21st century, Bill Wiley, the director of the Pacific Northwest National Laboratory (PNNL), and his senior management drafted plans for a unique new research facility. This facilty, which was intended to be one of DOE's scientific-user facilities,[1] would bring together the scientists and capabilities needed to understand, at a molecular level, the physical, chemical, and biologic processes critical to DOE's environmental missions especially the cleanup of its former weapons-production sites (for example, the Hanford site adjacent to PNNL in southeastern Washington state and the Savannah River site in southeastern South Carolina). On October 1, 1997, this facility, now called the William R. Wiley Environmental Molecular Sciences Laboratory (EMSL), began operation under the sponsorship of DOE's Biological and Environmental Research program. It brings a new level of research capability to bear on the environmental problems facing the DOE.

To address DOE's complex environmental problems, scientists in the EMSL will capitalize on the continuing revolution in the experimental, theoretical, and computational molecular sciences. Using a multidisciplinary approach involving chemists, materials scientists, geochemists, structural and molecular biologists, computer scientists, and applied mathematicians—experimentalists, theoreticians, and computational scientists-resident EMSL staff and visiting scientists will conduct research in

- The molecular sciences, to provide the fundamental understanding of complex environmental systems necessary to address DOE's environmental problems.
- Biogeochemistry and chemical processing, to focus this new knowledge on specific environmental problems.
- Computer science and technology, to provide the infrastructure needed to support widely distributed collaborative-research teams.

The research carried out in the EMSL in the next decade can be expected to have a profound effect on the understanding and solution of the environmental problems that confront DOE, this nation, and the world.

RESEARCH CAPABILITIES IN THE EMSL

The complement of research equipment and laboratory infrastructure in the EMSL can support a broad multidisciplinary effort in the environmental molecular sciences. Three major facilities house specialized and unique capabilities:

- The High Field Magnetic Resonance Facility, which houses a 750-MHz nuclear magnetic resonance (NMR) spectroscope, 2 600-MHz NMR spectrometers; and numerous 300-, 400-, and 500-MHz NMR spectroscopes. In addition, as of the middle of 1998, we have operational a 900-MHz NMR spectrometer, which was built by Oxford Instruments. These instruments support work in structural biology, interfacial chemistry, and microimaging.
- The High Field Mass Spectrometry Facility, which houses the world's first 11.5-tesla Fourier-transform–ion cyclotron resonance (FT–ICR) mass spectrometer, 7-tesla and 3-telsa FT–ICR mass spectrometers, and a number of other advanced mass spectrometers. These instruments support a wide range of activities, including combinatorial chemistry and gene sequencing.
- The Molecular Science Computing Facility (MSCF), which houses a 512-processor IBM Scalable POWER

Parallel computer system, a 20-terabyte EMASS hierarchical data-storage system, a state-of-the-art graphics and visualization laboratory, and systems that incorporate IBM's next-generation multiprocessor technology. In addition, the users of the MSCF are supported by a new generation of molecular-modeling software that takes full advantage of the computer systems.

In addition to the above major facilities, the EMSL offers, in 1 setting, a comprehensive collection of state-of-the-art equipment for research in the environmental molecular sciences. The capabilities embodied in this collection can be integrated as needed to address fundamental problems in 4 major categories:

- Nanostructural materials, for the design and synthesis of model materials for environmental studies.
- Interfacial structures and composition, for studies of the composition and structure of interfacial regions.
- Reactions at interfaces, for studies of chemical and radiologic processes at interfaces.
- Gas-phase monitoring and detection, for the development of new techniques for detecting and monitoring molecular species.

As one of DOE's national scientific-user facilities, the EMSL makes its research instruments available to researchers worldwide for studies in the environmental molecular sciences or related disciplines of national importance. For additional information on how to apply for time on the research instruments in the EMSL, visit our Web site (http://www.emsl.pnl.gov/).

RESEARCH IN THE ENVIRONMENTAL MOLECULAR SCIENCES

I cannot do justice here to the wide range of research being carried out in the EMSL. I will therefore pick a few examples on which to focus. I will use them to illustrate 3 of the major research themes in the EMSL: remediation of contaminated soils and groundwater, adverse health effects of exposure to hazardous chemicals, and processing of high-level wastes.

SINGLE-MOLECULE MICROSCOPY AND THE BIODEGRADATION OF CHLORINATED HYDROCARBONS

The most-prevalent class of contaminant on DOE's weapons-production sites is not, as one might suppose, radionuclides, but rather chlorinated hydrocarbons (CHCs).[2] In fact, CHCs—because of their widespread use as solvents, degreasers, and so on—are estimated to constitute a large fraction of the inventory of chemical pollutants in the United States. It has proved very difficult to remediate CHCs. However, a few strains of microrganisms have been found that slowly degrade them, and some of the enzymes responsible for the dechlorination steps have been identified. Deeper insights into the enzymatic processes involved in degradation will be necessary before we will be able to understand the nature of the rate-limiting steps and, if possible, develop or stimulate more-efficient processes.

For several years, Xiaoliang (Sunney) Xie and his collaborators in our laboratory have been developing methods to study single-molecule spectroscopy and dynamics.[3] Single-molecule sensitivity has been achieved at room temperature by using a combination of a laser, an inverted fluorescence microscope, and a photon-counting apparatus. The single-molecule measurements have provided unique and detailed information on condensed-phase systems, especially biologic systems. For measurements of single enzyme molecules, the molecules are immobilized in an agarose gel containing 99% water in which the substrate molecules are still mobile. A dilute solution is used to ensure that there is only enzyme molecule in the laser focus field.

For the last 2 yr, Xie, H. P. Lu, and L. Xun have been studying the enzyme responsible for the dechlorination of pentachlorophenol (PCP), pentachlorophenol-4-monooxygenase[4] (which has also been found to degrade trichloroethylene, one of the most-abundant contaminants on DOE sites). The active site of this enzyme is flavin adenine dinucleotide, which is fluorescent in its oxidized state. When the enzyme oxidizes the CHC, it gains an electron, and in this state it does not fluoresce. Eventually, the monooxygenase loses the extra electron, and the catalytic

cycle starts anew. Because of this property of the flavoenzyme, Xie and his co-workers have been able to follow the enzymatic reactions of a single molecule, similar to monooxygenase (PCP), in real time and directly measure the rate of the enzymatic reaction (usually referred to as the turnover rate in a catalytic process). The turnover rate is 100 or so per second.[5] Similar measurements are now being conducted on PCP to determine the turnover rate of PCP directly.

As important as that observation was, there is more to the story. When Xie and co-workers placed the dechlorinating enzymes in their native state in the agarose gel, they expected to see a steady fluorescence. Instead, they found that the spectral mean of the fluorescence flucuates with time and on the same time scale as the dechlorination process. This phenomenon is hidden in ensemble-averaged measurements. Xie and co-workers believe that the spectral variations are due to conformational changes in the enzyme and that these results confirm the earlier observations of Xue and Yueng[6] at Iowa State University. Using a single-molecule technique, those authors showed that the activity of identical copies of enzymes varied by over a factor of 4. They speculated that that might be due to conformational changes in the enzyme and that each conformation might have a different reactivity. If verified in real-time observations, this will be a major advance in our understanding of the detailed mechanism of enzymatic reactions. Clearly, single-molecule microscopy will be an important technology as we try to understand the bioremediation of recalcitrant environmental contaminants.

NMR SPECTROSCOPY AND THE STRUCTURE OF REPAIR ENZYMES

The EMSL has made a major investment in NMR spectroscopy. There are many applications of NMR spectroscopy, but I will discuss only 1 here—structural biology. In our studies of the biodegradation of contaminants, it is often critical to know the structure's key enzymes to be able to understand the mechanism of the degradative process. Similarly, the structures of the biomolecular species involved in the repair of DNA damaged by exposure to hazardous chemicals are critical to understanding the detailed molecular processes involved in the sequence of events that lead from exposure to uptake to damage to cancer (or other adverse health effect). Fourth-generation NMR spectroscopy provides the resolution equivalent to 2–2.5 Å X-ray spectroscopy. In addition, it does not require the protein to be crystallized, an often-daunting problem itself, and the species can be studied in aqueous solution, a far more natural setting than the crystalline environment.

The initial focus of the research effort in the EMSL is on understanding of the molecular processes involved in nucleotide excision repair (NER) of damaged DNA. NER is the process most commonly associated with damage resulting from exposure to carcinogenic chemicals. It involves several steps, such as unraveling of the DNA, location of the damage, excision of the altered nucleotides, and resynthesis of the correct DNA sequence, and each step can involve proteins with well-defined roles. The logical place to begin is with an understanding of the underlying structural basis of the initial recognition event and with the first structural signal to the proteins involved in the DNA-repair process. In NER, recognition of DNA damage is the responsibility of the protein XPAC (xeroderma pigmentosum group A complement). XPAC then recruits RPA (replication protein A) to the damaged site, and both are involved in recruiting other proteins: XPA recruits a heterodimer of ERCC1 (excision-repair cross-complementing protein 1) and xerodoma pigmentosum group F protein, which acts as a 5´ endonuclease, and RPA recruits xerodoma pigmentosum group G protein, which serves as a 3´ endonuclease. Together, the 2 endonucleases excise the damaged DNA patch in preparation for replacement with newly synthesized DNA.

To begin, Buchko and Kennedy determined the structure of a synthetic 40-residue peptide corresponding to the zinc-binding core of XPAC.[7] They have now completely assigned the backbone of the "minimal (damaged-DNA) binding domain," referred to as MBD,[8] and completely determined its secondary structure, including its topology. The residues in the zinc-binding domain of XPAC and GATA-1 (an erythroid chick transcription factor) make contact with the major groove in DNA. GATA-1 contains several hydrophobic side-chain amino acids that allow it to recognize a thymine-rich DNA sequence and bind tightly to it through the formation of several hydrophobic patches. XPAC, on the other hand, contains mostly hydrophilic residues. Kennedy and co-workers speculate that the zinc-binding domain of XPAC retains its ability to interact with double-stranded DNA but that its distribution of amino acids is such that it does not bind tightly to any specific DNA sequence—which would be a fatal flaw in an enzyme responsible for detecting DNA damage.

Although Kennedy and co-workers are studying XPAC, their goal is to determine the structure of XPAC–MBD bound to damaged DNA. Determining the full structure of the MBD of XPAC is certainly within range of the 750-MHz NMR spectrometer now in operation in the EMSL. However, determination of the structure of the complete XPAC could well require the 900-MHz NMR spectrometer. Studies of the complexes of XPAC with DNA or other proteins will almost certainly require the increased sensitivity and resolution of the 900-MHz instruments.

COMPUTATIONAL MOLECULAR SCIENCE AND MATERIALS FOR SEPARATING RADIONUCLIDES

The production of plutonium during the Cold War generated about 10×10^3 gal of high-level radioactive waste, which is currently stored in large underground tanks at the Hanford and Savannah River sites. The high-level wastes are complex mixtures of highly radioactive elements, such as cesium^{-137} and strontium^{-90}; long-lived radionuclides, such as americium^{-241}, plutonium^{-239}, and technetium^{-99}; and RCRA-listed chemical wastes, such as heavy metals, solvents, chelating agents, and nitrates. Cleanup costs could be sharply reduced if the small fraction of radioactive material (about 10–100 g per metric ton of waste) could be separated from the enormous volume of low-level waste, inasmuch as the former is destined for storage in an expensive underground repository where it must be immobilized over geologic spans of time (10,000 yr or more).

A number of metal-separation processes (such as solvent extraction, supercritical fluid extraction, liquid membranes, and ion exchange) involve complexing of the targeted metals with organic or inorganic ligands. For optimal performance, the ligand must satisfy a number of constraints, each of which is challenging but all of which together present a daunting exercise in materials design. When I joined the EMSL project in late 1989, Dave Feller and I examined the use of computational modeling techniques to help design a new generation of separation materials that would improve on the crown ethers that were being considered for extraction of cesium and strontium from tank wastes. We concluded that, with the computational resources available at the time, it was not feasible to address even the simplest—predicting the preference of 18-crown-6 for the potassium ion over the other alkali ions (lithium, sodium, rubidium, cesium, and francium). In fact, attempts to carry out such calculations met with failure.

Fortunately, advances in computer technology (processor speed, memory density, and disk capacity) continued unabated during the early 1990s, and by 1994 Glendening and co-workers[9] were able to carry out first-principles quantum-mechanical calculations on a realistic model of the exchange reaction:

$$K^+(\text{18-crown-6})_{aq} + M^+_{aq} \rightarrow M^+(\text{18-crown-6})_{aq} + K^+_{aq}$$

M^+ refers to any other alkali ion. The calculations, which took months to do on the computers available at DOE (a Cray C-90 supercomputer at the National Energy Research Supercomputing Center), predicted that the reaction was uphill (endothermic) for all ions $M^+ \neq K^+$, roughly reproducing the experimental data on endothermicity. More important, they showed that the preference for K^+ over the other alkali ions was the result of a subtle interplay between the solvation of the ion by water molecules and its "solvation" by the crown ether. The calculations also provided a wealth of important new information on the structure and binding energies of the gas-phase M^+(18-crown-6) complexes.

This experience, as well as others, convinced us of the need to develop a new generation of molecular-modeling software that could take advantage of massively parallel computer architectures. Parallel computing is the most feasible way to obtain the substantial increases in computing capability needed to tackle the more-complex molecules relevant to the separation of radionuclides, but it has posed a substantial challenge. In the early 1990s, many fundamental issues related to the design of massively parallel computers had yet to be settled. In addition, the explicit handling of communication required for massively parallel computers forced the scientific-software developer to manage the data used and generated in the calculation explicitly—a daunting task when dealing with the million lines of FORTRAN code typical of state-of-the-art molecular-modeling packages.

To tackle this problem, we assembled a team of theoretical chemists (Robert Harrison, Ricky Kendall, Martyn Guest, Jeff Nichols, Tjerk Straatsma, Michel Dupuis, and Tony Hess), computer scientists (Jarek Nieplocha and

Matt Rosing), and applied mathematicians (Rik Littlefield and George Fann). The group first designed the overall architecture for the molecular-modeling software, which is now known as NWChem.[10] The software was designed to be highly modular, with many of the "services" needed by the molecular-modeling applications, such as the computation of integrals over basic functions, being provided through application programmer interfaces (APIs). The extensive use of APIs is critical: It allows new techniques to be introduced into NWChem with minimal effort. In addition, NWChem uses a hardware-abstraction layer—the software-development toolkit consisting of global arrays, CHEMIO, memory allocator, and run-time library—to provide isolation from the computer hardware; only the software-development toolkit must be ported from one type of machine to the next. The net result is a molecular-modeling package that provides high performance, is scalable to hundreds if not thousands of processors, and is portable over a wide range of computer architectures (IBM SP, Cray T3D and T3E, Kendall Square, Intel Paragon, and even a collection of work stations).

Harrison and co-workers have carried out calculations on a derivative of 18-crown-6 synthesized by Li and Still.[11] The selectivity of this crown ether can be "tuned" by changing various functional groups in the molecule, but at the price of a far-more-complex molecular structure. It is not feasible to do high-level calculations on this molecule with a conventional supercomputer, and even the calculations reported here, whose aim was to quantify the scalability of NWChem, rather than to obtain highly accurate results, would pose a substantial challenge. For a single processor of the IBM Scalable POWER Parallel System (POWER2 processors), nearly 400 h of computer time is required for the solution of the matrix Hartree-Fock (HF) equations (899 basis functions using the aug-cc-pVDZ basis set[12]). With 180 processors, less than 1 h is required; that is, the use of 180 processors speeds up the calculation by a factor of 400! This phenomenon is called superlinear speedup and is caused by increase in the total amount of memory and local disk storage associated with the increase in the number of processors. For solving the HF equations, NWChem uses an adaptive algorithm that takes advantage of the additional disk storage, becoming more and more effective as the amount of available local disk storage increases. With the next-generation IBM computer (POWER2 SUPER processors) just installed in the EMSL, it is estimated that the same calculation can be done in 15 min. As can be seen even in this rather simple example, the developers of NWChem pioneered many innovations to take advantage of the power offered by massively parallel computer systems. NWChem greatly expands the range and accuracy of modern molecular-modeling approaches and will have a large influence, not only in environmental molecular science, but also in chemistry, materials science, and molecular biology.

CONCLUSIONS

DOE has made a commitment to remediation of its sites—a commitment that recognizes the effort as a formidable, but achievable, endeavor. By constructing and equipping the EMSL at the PNNL, DOE has dramatically enhanced its existing technical base, supporting development of the new knowledge required to clean up its sites and providing a powerful new resource to assist the scientific community in meeting the nation's environmental challenges. The resulting effort will lead to the development of technologies that span the full range of our environmental needs—from remediation of existing hazards to prevention of future environmental insults.

Research in the EMSL is now supported not only by the Office of Energy Research, but also by the Office of Environmental Management, primarily through the Environmental Management Science Program. The major focus of this work is on the molecular processes involved in the processing and storage of high-level wastes, the cleanup of contaminated soils and groundwater, and the health effects arising from exposure to hazardous chemicals. In the short term, this combination of basic, directed basic, and applied research can be expected to greatly enhance our understanding of the issues associated with the remediation of DOE's sites. In the long term, it can be expected to lead to the development of the new technologies that will be needed to achieve DOE's oft-stated goal of "better, faster, cleaner."

The EMSL will also help to forge the emerging scientific discipline of environmental molecular science, a multidisciplinary science that is still in its infancy. By drawing scientists from the chemical, materials-science, geoscience, structural-biology, and molecular-biology communities to the molecular-level issues that underlie environmental remediation, waste processing and storage, and human-health effects, it should be possible to

develop a sound strategy for addressing the nation's environmental problems. Research in the molecular sciences will also have far-ranging effects on many other DOE missions, including energy efficiency and national defense. Also, the new approach to collaborative research embodied in the EMSL—which makes full use of the latest advances in computing and communication technologies—will serve as a model for focusing the country's scientific resources on other vital national issues.

ACKNOWLEDGMENTS

I thank Sunney Xie, Michael Kennedy, David Feller, and Robert Harrison for providing the research material discussed here. I dedicate this paper to the memory of William R. Wiley, for his vision and steadfastness, and to Michael L. Knotek, for teaching us how to identify and cope with the myriad issues in establishing the EMSL. Without them, there would not have been an EMSL.

"Ultimately a hero is a man who would argue with the gods,
and so awakens the devils to contest his vision."

(special preface to the 1st Berkeley Edition of *The Presidential Papers* [1976]). I also dedicate this article to the staff of the William R. Wiley Environmental Molecular Sciences Laboratory, in whose hands the future of the EMSL rests.

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Discussant

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The importance of subsurface bacterial transport in the spread of waterborne diseases has long been recognized. Indeed, the first indirect evidence of subsurface bacterial transport occurred in 1854, when it was observed that a cholera epidemic in central London was the result of bacterial contamination of a public well.[1] Injection and recovery tests involving the direct addition of bacteria to freshwater aquifers were conducted as early as the 1890s.[2,3] The purpose of early additions of bacteria was to provide a convenient tracer for following the movement of groundwater through karstic, or fractured-rock, terrain. Indicator bacteria, such as coliforms, have been added to the subsurface since the 1930s[4] to investigate the transport potential of pathogenic bacteria near water-supply wells. Only within the last 10 yr have specific populations of bacteria been added to aquifers to enhance the degradation of subsurface contaminants. However, many current mechanistic studies of subsurface bacterial transport are being carried out to learn more about the feasibility of using nonindigenous or waste-adapted bacteria in the cleanup of subsurface contamination.

IMPORTANCE OF SUBSURFACE BACTERIAL TRANSPORT AT DEPARTMENT OF ENERGY SITES

Subsurface bacterial transport is potentially important for engineered remediation and natural attenuation at various contaminated sites at Department of Energy (DOE) facilities, especially those subject to subsurface contamination with organic chemicals. The role of bacterial transport at such sites can be complex and can involve various effects, such as the following:

- Seeding of contaminated zones with bacteria that are genetically engineered for or have adapted to a particular contaminant.
- Facilitated transport of hydrophobic or surface-active contaminants by mobile bacteria, which might enhance the spread of these chemicals.
- Cotransport of bacteria with dissolved organic contaminants, which results in longer contact between the contaminants and the populations that affect their degradation.
- Increased dissemination of genetic information, particularly if introduced bacteria carry genes that confer more-efficient breakdown of organic contaminants.

Little is known about the last 2 mechanisms, and more information is needed about all 4. More research on the role of bacterial transport in the ecology of subsurface microbial communities that ultimately degrade contaminants is also needed.

FUTURE RESEARCH CHALLENGES

Geohydrologic Complexities

Many of the field experiments in which labeled bacteria were injected directly into an aquifer with a conservative tracer (nonreactive solute) involved rather homogeneous sandy deposits.[5–7] In well-sorted sandy deposits, the bacteria injected into the aquifer are assumed to follow the same flow paths as the conservative tracer. Therefore, the conservative tracer can be used to predict what points in the acquifer need to be sampled in order to capture breakthrough of labeled bacteria. Also, the concentration histories of the conservative tracer provide important information for the hydrologic portion of a predictive transport model and allow for a reasonable estimate of bacterial retardation.[8]

However, most contaminated DOE sites are characterized by substantial heterogeneity that can involve fracture-flow or adjacent strata that vary in hydraulic conductivity by a factor of several powers of 10. In heterogeneous aquifers dominated by preferential flow paths, bacteria can be excluded on the basis of size and porosity from substantial fractions that are accessible to the conservative tracer. Injection and recovery investigations involving fractured-rock aquifers suggest that transport of bacteria in fracture-flow environments can be much faster than that of the conservative tracer.[9] That phenomenon has also been demonstrated in the laboratory[10] for highly stratified formations in which the major conductive zone is next to a layer characterized by porosity too fine for access by microorganisms.

On the larger scales that are typically involved in natural attenuation or engineered restoration of contaminated aquifers, even the relatively homogeneous sites look heterogeneous, at least for the purposes of predictive modeling of bacterial transport. It is evident that the simple deterministic models that are typically used to describe small-scale (<10 m) bacterial transport in saturated porous media are not adequate for larger-scale application at most, if not all, contaminated DOE sites. That is, in part, because of the aforementioned geohydrologic complexities that often become manifest at larger scales. Bacterial transport models are needed that can account for physical variability in aquifer structure, perhaps in a manner consistent with the application of stochastic theory to describe mathematically the large-scale (>100 m) movement of conservative solutes in sandy aquifer sediments.[11]

Reconciling Discrepancies Between Field and Laboratory Results

Much of the detailed information about bacterial transport behavior in saturated granular media derives from flow-through column experiments involving repacked subsurface material. A number of the more-recent studies on this subject were sponsored by DOE.[7,12–14] Typically, filtration theory is used to describe the removal of unattached bacteria being advected downgradient through granular media. In the filtration model, a bacterium's affinity for attachment to the grain surfaces that it comes into contact with is quantified with a collision efficiency (a) factor,[5] whose value can vary from 0 to 1.[15] Recent findings from flow-through column experiments involving bacteria[7,12–14] and bacteriophage[16] transport through representative granular media under reasonable chemical and hydrologic conditions suggest little microbial mobility and collision efficiencies of 10^{-2} to 10^{0}. In contrast, recent field observations of bacteria[5,17] and bacteriophage[18] transport through aquifer sediments suggest substantial mobility with corresponding collision efficiencies of 10^{-4} to 10^{-2}.

The discrepancy between field and laboratory results can be explained, in part, by the destruction of pore structure when subsurface soil is repacked into columns. It has been demonstrated that, when intact subsurface soils are repacked, bacterial transport is greatly diminished because of the destruction of preferential flow paths.[19] It has also been shown that in situ transport of bacteria through undisturbed aquifer sediments can be different from that in core material taken from the same location.[20] A major advantage of flow-through columns is that they offer a much greater degree of control over experimental conditions. However, the apparent discrepancies between field and laboratory results, and the growing recognition that processes that control subsurface microbial transport behavior can be interrelated and operate on spatial and temporal scales that are not conducive for laboratory study, emphasize the need for more in situ transport studies. Accurate assessment of the potential role of bacterial transport in the restoration of contaminated groundwater at many DOE sites might require injection-and-recovery experiments on scales that are larger than those of previous studies. However, because much of the emphasis over the last 2 decades has been on flow-through column experiments, development of methods for conducting large field-scale investigations of subsurface bacterial transport has lagged.[8]

Biologic Factors

Most of the previous research on the controls of subsurface bacterial transport has focused on physical and chemical factors. Immobilization at stationary surfaces is a major determinant of the extent to which bacteria move within aquifers. However, many of the other important determinants of a bacterium's fate and transport in aquifers are biologic,[21] and the biologic controls of subsurface bacterial transport have been largely ignored. Although protozoa are known to be common features of both shallow[22] and deep[23] groundwater habitats, their role

in the transport of bacteria through aquifers has not been well studied. These eukaryotic predators of bacteria in the subsurface appear to be particularly abundant ($\geq 10^5$/g dry weight) in organically contaminated aquifer sediments.[24,25] At least one organically contaminated sandy aquifer yielded evidence that protists might be more efficient at removing unattached bacteria being advected downgradient than are the organic and mineral coatings on the sediment grains.[26] Also, growth of bacteria being transported downgradient in organically contaminated aquifers can be substantial and can offset the losses that occur as a result of attachment.[21]

The dearth of information on the importance of biologic controls on subsurface bacterial transport is due largely to the fact that many aspects of the subsurface ecosystem are so poorly understood that their effects on bacterial transport are not fully appreciated. A number of the biologic controls are interrelated and difficult to describe mathematically; this makes their study in field experiments difficult. However, the effects of several factors, such as microbial competition, predation, parasitism, and growth, on subsurface bacterial transport are ideal candidates for studies sponsored by the Biological and Environmental Research program that could build on the subsurface ecologic information already collected in previous research.

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Discussant

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My theme is diversity. Dr. Knotek talked about the hyperthermophiles and about the thermophiles as sort of the center of the tree. But at the opposite end of microbiology are organisms that live in the cold. I will show you a sample site that we work with to look at cold-loving organisms. We worked with a group of Russian scientists who specialized in permafrost soil. There were five sampling sites in a region of northeastern Siberia right off the Arctic Ocean, in the Kalama region near the East Siberian Sea. One of the 2 regions of the world that geologists think has been permanently frozen from the time that the sediments were laid down. In principle, given the depth of these sediments, we can take samples out of the freezer of microbial communities that lived there some 3 million years ago.

We can extract a considerable amount of DNA from these samples. We can see many organisms in these samples varying in size and number in different sites. We stained them with a fluorescent protein stain. They look to be perfectly healthy and are particularly numerous at these sites. We can isolate a few of what we suspect to be there on the basis of the DNA. It is not surprising that if an organism has lived a million years at –12°C, it will not grow when suddenly put at maybe 10°C.

Our interest is in learning more about the physiology of these organisms. How do they adapt to that environment? In particular, how do they live for such a long period at such low temperatures? The point is that the cold-loving organisms are of interest, as well as the high-temperature organisms.

We determined the 16-S ribosomal sequence for the DNA extracted from these samples. There was a gram-positive organism that would be similar to the living organisms that we know in the arthrobacter group. But we also saw some gram-negatives. What are the gram-negatives like? If we determine the sequence and compare it with sequences of other organisms, we can see that they fall into clusters. One cluster includes an organism—recently isolated by some people in our group—that oxidized iron. This represents a primitive lifestyle of iron-oxidizing organisms and might have relevance to the recent Martian discoveries as well. That is one example of new diversity of physiologic types.

What generates the high diversity that we find in nature? We extracted DNA from soils of different depths—surface soils, the unsaturated subsoil, and sediments below the water table. The samples were from 2 sites, in Virginia and in Delaware. This work was done collaboratively with Oak Ridge National Laboratory and partially funded by Office of Biological and Environmental Research.

We amplify the 16S genes, cut them with restriction enzymes, and look for different patterns that indicate different genotypes. When we compare the different patterns, nothing is dominant. We looked at some 700 clones from only 5 g of soil; virtually every organism is different. That is a surprising pattern in biology because organisms that compete invariably show dominance—the dominant organism wins. If we go down to the saturated zone, we see dominance. Organisms are winning the competition. But in the saturated zone, organisms can move, as Ron Harvey described, and substrates are equally mobile.

The interesting pattern, because it is unusual, is the top pattern. The unusual soil diversity profile occurs particularly in the top one which we would call a noncompetitive diversity profile; there is a lack of dominance.

In ecology, the hypothesis that explains a noncompetitive profile would include a superabundance of resources, which is unlikely in these soil communities. Resource heterogeneity is a possibility. But modeling studies suggest that the explanation lies in spatial isolation. In these nonsaturated zones, the organisms are spatially isolated. They are living in their own world and therefore are not competing. That has important practical implications if the spatial-isolation hypothesis is supported. It is easier for an alien population to colonize but more difficult for it or any population to become dominant. That indicates something about bioremediation and the expected rates of bioremediation. Overall, biodiversity should be readily preserved in such environments because there are infinite protected niches for those organisms.

Soils should and seem to harbor a huge amount of microbial diversity. The estimates suggest some 10,000 species of bacteria per gram of soil.

Finally, microbial endemism should be the rule, that is, everything is not everywhere. In other words, you have uniquely, locally adapted species, which has never been thought to be the model to apply to microbiology. The spatial-isolation hypothesis has a lot to do with our understanding of biodiversity in microbial communities.

To conclude with an application of biodiversity, I note a case in which nature has not made an organism to do a job that we would like done—to grow on polychlorinated biphenyls (PCBs). Nature has not combined the complement of genes to allow organisms to grow on PCBs. If such an organism did exist in nature, it would try to metabolize PCBs, producing an acyl hyaline compound, which would be extremely toxic and kill the organism immediately. If there were an organism that could grow on PCBs, it would be suicidal.

We have tried to remove chlorines by dehalogenation to avoid ending up with an intermediate that forms a toxic product and then to look to nature for dehalogenase genes that might be useful. We have 3. One is the PCB pathway, which leads to the potentially toxic intermediate. Of the other 2, 1 is a hydrolytic gene, and the other is a reductive dechlorination gene. An oxidative gene, if added to the projected intermediates coming from PCB metabolism, would produce nonchlorinated products, and the organism should grow. And, in fact, we can get organisms to grow on some PCBs.

In one example, we introduced 2 genes into an organism to begin to co-oxidize PCBs. One organism grew on 2-chlorobiphenyl, which is one of the most important congeners after reductive dechlorination of PCBs. And one grew on 2, 4-dichlorylbiphenyl, another important congener from reductive dechlorination. This shows how we can use diversity in nature—in this case, different genes that we put together in an organism—to achieve growth.

How might we implement this? One of our collaborators, Walt Weber, at the University of Michigan, is working with slowly mixed auger systems. The soil remains in situ and is not constantly mixed, but only stirred occasionally. You can move over a site, mix the soil down the stem of the auger, put in organisms or nutrients, and let the organisms do their job. This is an example of the use of diversity in bioremediation.

Part IV

Science Policy and Societal Impacts

Science Policy and Society: Current Challenges, Future Priorities

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Berkeley, California

In the 21st century, biologic science will occupy the same role that physical science occupied in the early 20th century. In looking toward the 21st century, we have to address a number of important topics:

- Whether support of basic science should or can be viewed as a wise investment strategy.
- What the changing balance of private and public funding of science means.
- What the ethical, legal, and social issues in science are.
- How we will educate and inform the public about science.
- What the roles of basic and applied science in mission-oriented agencies will be.
- How R01 grants versus "big science" and team research will work out.
- What the role will be of the Biological and Environmental Research (BER) program in the Department of Energy (DOE).
- How the national laboratories will work in preserving the nation's scientific and technologic competitive edge.

Should or can support of basic science be viewed as a wise investment strategy? An article in the *New York Times* ("Study finds publicly financed science is a pillar of industry," William J. Broad, May 13, 1997) describes a study that found that publicly financed science is the "pillar of industry." The study, conducted for the National Science Foundation by a private research group, found that 73% of the main science papers cited in American industrial patents in 2 recent years were based on domestic and foreign research financed by government or nonprofit agencies. Private companies paid for the rest. Thus, publicly financed science had turned into a "fundamental pillar" of industrial advance. That finding sharply contradicts the view that publicly financed research, which gave birth to high-technology industry, is no longer so important. The article quotes Charles F. Larson, executive director of the Industrial Research Institute, a nonprofit group in Washington that represents large companies: "[The report is] going to make people realize something that they should have known all along—that public investment in academic science through government-funded programs, pays dividends to society . . . that it pays off handsomely." I could not have said it better. I think that some of the members of this panel could also address this. But it seems that the case is very well made that it is singularly important for government and tax money to be used to develop fundamental knowledge because it pays back to society.

As to the ethical, legal, and social issues in science, I am the president of the American Society for Cell Biology (ASCB), and we took our charge quite literally and went on record with a statement about the science of

cloning of sheep. It is important that scientists police themselves. ASCB went on record that, indeed, we do not approve of cloning of human beings and that we do not know enough to allow going forward in that direction. But we also have gone on record to make sure that the fear of cloning, which can be exaggerated, is not going to prevent the magnificent scientific advances that the scientific community is making, including the use of DNA-engineering and cloning techniques, which play an important role in scientific advance.

David Cox addresses this issue with regard to the ethics of the Human Genome Project. Charles Shank addresses the role of basic versus applied science in mission-oriented agencies, but I want to address the question of R01 grants versus "big science" and team research. This theme has interested many of us in industry, in national laboratories, and in universities. There seems to be a question of individually driven science as opposed to team science. It should not be an either-or situation; it should not be one versus the other. Indeed, it is important to make smooth boundaries and connections between individually driven science and big science.

The national laboratories and the Office of Biological and Environmental Research (OBER) are capable of advancing magnificent, team-driven big science. But it is important to recognize that it is individuals who actually do it. We need to bring multiple fields to bear on large problems, and we must use the best talents and the most-original intellects to achieve our goals. Few of us would question that it is important to have the best and the brightest in the national laboratories and in OBER-related science to achieve the goals that we want to achieve.

I want to say a couple of words about the role of the OBER program in DOE and about the future role of the national laboratories in the nation's scientific and technologic competitive edge. This will be somewhat personal. In the early 1980s, I was doing a sabbatical in England, and I was asked to become a charter member of the secretary of energy's Health and Environmental Research Advisory Committee (HERAC). When we walked into the room, there were 10 or 12 university and industry people and 2 or 3 scientists from the national laboratories. The committee had been created to take a hard look at the quality of the science supported by the Office of Health and Environmental Research (OHER) of DOE. However a number of the members unofficially wanted to do away with the office. The questions always were, Why should biology be done in the national laboratories? Should they be allowed to do it? These members felt that we were going to look at the OHER program and would come to the conclusion that the science was not good and thus the program should be closed. I now would like to share with you an excerpt from the committee's report. This is about 10 yr old, but it is still relevant. I was delighted to be a member of HERAC and help to write it.

> "Throughout all of our discussions, the themes came up again and again about the unique role that OHER plays, about the breadth and the coherence of the Program, about the importance of the field laboratories as national resources, about the critical tightness of funding, and about the heavy losses to be expected if the pattern of diminishing funding continues. Regardless of discipline or distance from OHER, the members shared this common perception of OHER's role and its special value. We tried to write these perceptions into the document, but I wish we had some better way to transmit to the skeptics how a committee of virtual strangers came to such a common, strongly positive consensus.
>
> For those who control the future of OHER, we hope they can come to appreciate what we see in the Office's performance and style. Not only does the Office carry out its primary mandates effectively, but it has created, and is responsible for a very special national resource at its on-site laboratories. And perhaps most importantly, in its approach to problem-solving by supporting science broadly, OHER has come to play a critically important role in the cutting edge and even the survival of large pieces of relevant, modern, health and environmental research."

That is the case even today. People should look at what the OBER program (the current name of OHER) has actually achieved—the accomplishments of this group, that it takes risks, that it supports science for an extended period, and that important biologic findings have come to pass because of this pattern of thinking and daring.

A perfect example is the assay of Bruce Ames. The assay is now used everywhere and was funded by this office. He could not get National Institutes of Health (NIH) funding for it. Now it is established, and all government testing agencies use it.

The field of DNA repair would not be on the map were it not for this office and its vision. Many of the technologies—the flow cytometer, comparative genomic hybridization, all sorts of other exciting technology—that we now use and take for granted were funded by this office.

My own research, being very basic, was alien to how people normally think, and was regarded initially as something that could not possibly become important. It would have not been possible to do it without the support and vision of OHER. I have said this in front of many audiences: my very beginning was in the National Science Foundation, where someone was willing to give these ideas a chance. NIH committees looked at my first 2 grant applications and said that they did not see how it could work. They were unwilling to take any chances within the science. But 15 yr later, this exciting field of extracellular matrix signaling is on the map. Think about it: Without that 15 yr of funding, this field would not be on the map now. I want to say that I vote with my feet, as well as the feet of my colleagues, who have been there to be beneficiaries of this. That is why I am here today.

Where do we want to go in the 21st century? The ultimate mission of the health programs in OBER is related to risks to human health. Today, you have heard panels on global and atmospheric research, on the human genome, and on nuclear medicine; I am not going to reiterate them because they articulately discuss some of the exciting things that the science is going to bring. My field is health effects, and I thought that I would address that a little bit more. In the 21st century, the sequencing of the genome, which is already under way, will be a reality. What we must prepare for now is not only the structure-function relationship of proteins, but how they are regulated at the subcellular, cellular, and tissue levels. We need to understand function in the context of the organism. We need to address human biology and physiology and, most importantly, functional genomics and postgenomics; we need to address the complex problem of individual susceptibility and the cumulative effect of low-level environmental insults. These are very important challenges. We will meet these by bringing funding agencies together through the unique capabilities of academia and of the national laboratories, where many disciplines are brought to bear to answer such complex questions. That is why we need biology in the national laboratories—to bring many disciplines to bear on the complex problems of life and regulation of function and to use teamwork to answer the big questions.

Discussant

Mary Clutter
Assistant Director, National Science Foundation
Washington, DC

President Clinton made an important statement during his commencement address at Morgan State University in May 1997. He said that the 20th century was the age of physics and that the 21st century will be the age of biology. I would like to discuss why the 21st century will be the age of biology and how this came about.

There can be little doubt that this country is first in the world in biomedical science. This has happened over the last 50 yr. Actually, the major growth has occurred over the last 30 yr and has made it possible to say that the 21st century will be the age of biology.

A number of factors have contributed. A major one was that we placed emphasis on supporting individual investigators, especially young investigators, pursing their own ideas in competition with each other at the national level. (That has not been true in other countries, although the situation is changing somewhat.) One great benefit of our investment in biologic research over the last 50 yr is that we now have well-trained biologists everywhere in this country, not just at the top research universities. These scientists are also at the national laboratories, at so-called second-tier universities, at small colleges, and of course in industry. Thus, we have unsurpassed intellectual strength in biology everywhere in this country that can be tapped as we embark on the age of biology.

We must note that as we embark on the age of biology, we are also living in the age of information. What does this mean for biologists? It means that scientists can now work with and analyze immense data sets that allow them to look at information both temporally and spatially in ways never before possible. The data can be shared with other scientists in this country and all over the world. This capability is changing how we think about biologic questions, and it will certainly change how biologic research is conducted in the 21st century.

Let me give you a couple of examples. In January 1997, I had the good fortune to go to Antarctica and interact with scientists who are doing research there. They are in daily contact with their laboratories in the United States or whatever country they are from via the Internet. They can tap into any database available on the World Wide Web from that remote location.

In May 1997, some visitors from the Mongolian Academy of Science visited me. They want to participate in leading-edge biotechnology research because they now have access to the Internet. It is increasingly possible for their scientists to work with our scientists. In fact, it is happening already. The National Science Foundation (NSF) supports a number of long-term ecologic research (LTER) sites throughout the United States and Antarctica. We are now connecting scientists around the world in an international network. Scientists in Mongolia have begun to collaborate with our LTER scientists in Colorado. Thus, biologic research is changing. One does not have to feel isolated at some remote location. One can be very much in contact with the mainstream of biologic thinking from anywhere, and this will be increasingly possible as we move into the next century.

That also means that scientists anywhere can participate in large projects. Increasingly in biology, scientists will collaborate on large projects, such as genome projects, linked via the Internet. They will not need to work in teams at the same location as physicists who work with large accelerators need to do. They can work alone, but be connected with others anywhere in the world who are investigating the same problems. We will have to be prepared to support that mode of research.

It is increasingly clear that the major unsolved problems in science require support through the cooperative efforts of more than a single agency. That is certainly true for the Human Genome Project, but it is also the case with other projects. For example, the Department of Energy (DOE), NSF, and the US Department of Agriculture are supporting the Arabidopsis Genome Project, one of the most-important collaborative efforts in plant biology today. It links scientists all over the world in a multinational project with milestones and goals established not by federal administrators but by the scientists themselves. They set the priorities, and government officials act as facilitators. So far, it is working marvelously. However, we must be aware that that might cease when genes with commercial value are sequenced. For the moment, the coordinated effort is working.

Another cooperative program is the one on bioremediation. The partners are DOE, the Environmental Protection Agency, the Office of Naval Research, and NSF. It is a joint peer-reviewed competition open to investigators anywhere in the country. It is still too early to announce any outcomes, but some very exciting research is being supported because agencies have joined in partnership to support large projects that would be difficult for a single agency to fund.

A final example is the Protein Data Bank, supported by DOE, NSF, and the National Institutes of Health. It would certainly be too expensive for 1 agency alone to support. Yet the data are invaluable to scientists supported by the 3 agencies. As we move into the next century, I believe we will see an increase in the number of multiagency partnerships.

The President said something else at Morgan State that has not been quoted widely. He proposed that this country join with other nations to solve environmental problems. I see that as a huge, compelling challenge for our agencies as partners. Successful scientific partnerships, such as the Arabidopsis Genome Project, might well be used as models. But it is essential that scientists identify the questions and set scientific priorities and goals to construct a roadmap to the future that can be used by agencies as they set their priorities.

The 21st century will be the age of biology, but only if we use the talents of scientists from many disciplines, wherever they are, and the combined resources of agencies, public and private, in partnership.

Discussant

David Cox
Genetics Department
Stanford University
Palo Alto, California

I do not have a long history with the Department of Energy (DOE), although I have been involved with it in many ways. But I want to be a part of this celebration because of the Human Genome Project. Throughout my scientific and medical career, I have been trying to figure out how to use genetics as a tool to monitor health effects. I do not believe that it is the answer to life, but I do believe that it can be an important tool. I remember being at the Lawrence Livermore National Laboratory in the mid-1980s, listening to Charles Cantor talk about some guy named DiLisi, who thought that the human genome could be sequenced. Interested as I was in genetics, I did not have the vision to see what would be possible, and Charles DiLisi did. That vision revolutionized my scientific career, and that is why I am so pleased to be here.

Mina Bissell, our panel chair, gave us a charge, that is much broader than genetics and the genome project: science in the next millennium. Because I am a fairly narrow person, I am going to focus on that in the context of genetics. I too would like to refer, as Mary Clutter did, to President Clinton's May 1997 commencement speech at Morgan State University. The president stood up and said that the next age will be the age of biology. I do not know that much about Washington politics, but I do know that perception can lead to reality. If the president says that it is going to be biology, it is likely to be biology. How is biology going to go forward in the context of human health? I would like to use genetics as an example here and start with the Human Genome Project.

For a long time, people talked about using human genes to get a better understanding of disease, particularly genes that caused diseases when mutated. In the early 1980s, when this was suggested, some thought that it would never happen. But, slowly but surely, individual disease genes were cloned. The cystic fibrosis gene—the gene that was necessary and sufficient for this relatively rare genetic disease—was cloned. It cost at least $200 million to do that. It was one thing to stand up and talk about using modern molecular biology and genetics for human health, but even those as naive as I am with respect to economics knew that this was not in the cards if it was going to cost $200 million for each disease.

That is where DiLisi had great vision. The idea was that if one could get all of the genetic information in a rapid and cost-effective way, one could apply it to all the different diseases. The important insight was related to making that happen. Biology is as much of a nightmare as atmospheric science. Biology is difficult to model. I think that the whole world is difficult to model. But the advantage of genetics is that it is bounded by discrete chromosomes. The human genome has 3 billion base pairs. That is the bad news. The good news is that when you are up to 3 billion, you are finished.

Even when we reach that point, it does not mean that we are going to understand everything, but we will have a completed tool for unraveling biology. The important vision of the genome project is not that it will stamp out disease, but that it is bounded, it is doable, and it will provide a resource that can be used into the next century.

How does one sequence the genome? I do not believe that it will be done by individual small-laboratory investigators. We have tried that. That was the idea of cloning individual genes; it cost a fortune, and it was not efficient. But it does not require the same mass group of people that some of the very big, high-energy physics projects did.

Since 1990, my colleague Rick Myers and I have managed one of the National Institutes of Health Genome Centers. The group numbers 30. Most of the genome centers are not bigger than 50—smaller than most major research laboratories.

With respect to personnel, there are two ways to think about the genome project and big science. One is to assemble as many stupid people as you can so that they will not recognize how boring the task is. The other is to assemble few smart people. The latter is what has led to the success of the genome project.

I agree with Mina Bissell that what we need—in the context of the genome project, in which only a small percentage of the human genome has been sequenced when the goal is to have it finished by the year 2005—is a

select group of smart people. We already have smart people at the DOE laboratories, and they can make a major dent in sequencing the human genome and in making the reagents and technologies available to us.

This is an international collaborative effort, but not every laboratory in the world can put it together. It requires groups that are used to dealing with big projects, that are smart, and that know how to deliver the goods. This is one place where the Biological and Environmental Research (BER) program, in addition to having the vision, has the prospect of delivering the goods. The idea of a joint sequencing center, combining 3 of the national laboratories, is outstanding. It is timely, and it is necessary.

How is the genome sequence going to be applied to human health? The common way that is talked about, which is reductionist, is that we are going to find the gene that causes a disease, figure out how it works, and come up with a therapy for the disease. That is not practical in a realistic period of time. I am not getting any younger, and it is certainly not practical for my scientific lifetime. It would be like trying to model and understand the whole environment.

We will use the genetic information cost effectively by using the variation in the human genome as a way of satisfying human-health problems in terms of their common genetic cause. When I talk to physicians about this, they ask, Are you going to throw out all my diagnoses? When I talk to other groups, they ask, Do you think that genetics has the best answer? All genetics does is provide a type of information that enables one to take what looks like a single disease and group it into the 5 or 10 most-common forms on the basis of genotype.

It is simple to think about that. A treatment that works on only 20% of patients with a particular disease is not going to be a very effective treatment.

But genetics can be used to break that disease down into, say, 5 homogeneous entities, then perhaps 5 treatments would take care of everyone.

This does not have anything to do with gene therapy. It does not have anything to do with understanding how a particular gene works. What it has to do with is that the genome is bounded and that 1 in 1,000 base pairs in the DNA is variable, and you can use that information for a mix-and-match—a correlation.

I am absolutely sure that the genome will be sequenced, that we will have the variations, and that we will have the technologies and the chip arrays that are going to identify the variations. But I do not think that our society is set up to use the information. The DOE laboratories can play a major role in providing the information, but I suggest that the BER program could play a major role in making use of it with respect to human-health effects.

I am on 2 government groups. The Task Force on Genetic Testing has just finished its work. It was a task force of mixed public and private people—lawyers, economists, health-maintenance organization people, and ethicists—who tried to figure out how to deliver safe and effective genetic tests.

The task force asked several questions: How do you decide whether a particular test is safe or effective? Do we need to have laws and regulations to protect individuals' autonomy and individual freedoms? How can scientists use genetic information to make sure that people are helped more than they are hurt?

President Clinton, in his Morgan State speech, talked about ethics, particularly biologic ethics and how this biologic work was going to be for society. The president said that, if we are going to have this new technology, it should extend to all people in the United States, not just to a few rich people. It should be fair under law so that it does not discriminate against some people. It should help people more than it hurts them. This should sound familiar to physicians.

Are we in a position now, using genetic tests as an example, in which more people will be helped than hurt? Absolutely not. In fact, one way in which people can be hurt the most is in the inability to get health insurance. Much to my surprise, the president suggested that there be legislation to ensure that genetic information is not used to discriminate unfairly against people with respect to health insurance. I applaud that statement.

What the Task Force on Genetic Testing, much to its surprise recognized, is that the missing component is the continuing collection of the data needed to figure out whether a test is useful or harmful and which therapies work with what test results, and which do not. This is not done in our country now. We pretend that we use science to make policy decisions; in fact, we make policy decisions on the basis of our preconceived notions. If science helps to support those preconceived notions, we use it; if it does not, we say that it is bad science.

I would hope that in the 21st century we can use genetics as the paradigm and have all science policy be more evidence-based. If that is to be the case, at least with respect to human-health effects, the public will have the scientific facts and other information needed to decide whether it wants to use a test or not.

The President's Bioethics Commission, which I am also involved with, feels exactly the same way. That commission is dealing with the cloning of human beings. Rather than making moral statements, we are focusing on our lack of scientific information on this complex technology; we think that it would be a good idea to have a substantial knowledge base before generating human beings with this technology.

The BER program should be involved not only in sequencing DNA to drive biology but also in collecting information with respect to the human population to see how the genome sequence can be used to help and not harm. DOE can play an important role in filling that information gap.

Discussant

Charles V. Shank
Director, Lawrence Berkeley Laboratory
Berkeley, California

From my days in industry at Bell Laboratories, I know that a company like AT&T was able to invest in basic research as well as applied research, with the idea that it could capture the value of its investment because it essentially had a monopoly on telecommunications.

Today, very few businesses can own all of the investment in basic research in their fields. A country needs to build a foundation for doing basic research, as opposed to providing a financial driver for industries to be able both to invest and to recover their investments. In the United States, 73% of the applied industrial research has had some basis in or connection with federally funded basic research in universities and in national laboratories.

The United States has had a 50-year legacy of investment in research, and the technologic progress that has been made as a result of it is amazing. We are able to form businesses that cannot be found anywhere else in the world. Investing in research will probably be even more important in the next 50 years than it has been in the last 50.

As we look at the kinds of basic research that are going to go on, one question that arises is, What is the role of the Biological and Environmental Research program in the Department of Energy (DOE)? DOE is different from other funding agencies in that it is driven by problems—problems in energy and problems left by the legacy of wastes that have accumulated as a result of the Cold War. It is going to be more important than ever to apply biology and environmental-effects research for the technologies that will be developed to clean up this multi-hundred-billion-dollar mess that the country is involved in. Having biologists work in close connection with researchers in energy and in cleanup will be crucial for the effective and cost-effective kind of cleanup that will be needed.

The multidisciplinary laboratories, now called "multiprogram laboratories, have the capability of assembling expertise in a wide variety of scientific fields. We are often asked, How can you be a multiprogram laboratory if you do not have a sharp center of focus? I think that our focus needs to be to assemble teams to attack problems. Problems are seldom defined by a discipline or a focus. They are defined by a national need. The laboratories have shown themselves to be extraordinarily adept at building multidisciplinary efforts.

We can look at the sequencing of the human genome as an example of the problems facing DOE today. In the laboratories we have the skill and capability to bring engineers, physicists, and biologists together to invent a new kind of biology that is going to take advantage of advances in automation and informatics. Structural biology is another example. We are going to have biologists taking advantage of the new synchrotrons, the advanced photon source, and the advanced light source. The synchrotrons produce unique kinds of X-rays for doing structural determinations. It has taken, in many cases, months, sometimes years, to solve the structure of a complex protein. I predict that within the next 5 years we will be determining structures in fractions of a day, maybe even in minutes, as we begin to marry state-of-the-art computation, biologic needs, and advances in third-generation light sources.

As to the cleanup program, large areas in this county are carrying a load from previous use for nuclear materials. The legacy of that work is going to require not only an understanding of the chemistry and environmental effects under the earth, but also a bringing together of a biologic perspective, a computational perspective, a chemical perspective, and a physical perspective. Multiprogram laboratories can do that.

Discussant

Daniel Goldin
Administrator
National Aeronautics and Space Administration
Washington, DC

When I travel around the country, I ask executives and others, "Do you believe in the future dream of America? Do you believe that America is going to be a great country in the year 2025?" Almost invariably, they will think about it and make a little sarcastic comment, but eventually, they will say, of course we are going to be a great country. Then I say, "Who is responsible? Are you?" People have a very hard time in answering because, in the corporate world, you cannot go beyond 5 yr in your thought process. I have yet to see a corporate strategic plan that goes beyond 5 yr.

Of the basic research in this country, 8% is paid for by corporate America, and the lion's share is being left to the federal government that everyone talks about and wants to cut the budget of—they all go to the polls and vote for deficit reduction. But without federal funding for long-term research, cutting-edge research, it would be almost nonexistent in this country.

The federal budget is around $1.6 or $1.7 trillion, and $30 billion of it goes into nondefense R&D. Even adding all defense-related R&D takes it up to $70 billion a year, not a huge amount on a national scale. More and more, when one goes to Congress to testify, one hears, "What is it going to do for my constituents today?" The answer is probably very little. It will do something for their children. That is the timeframe we have to use because if you are doing long-term basic research and getting results in 2-3 yr that have an effect, you should not be doing it. At least this is one government bureaucrat's view of the situation.

American industry does a wonderful job in near-term product development in the 2- to 4-yr timeframe. But the timeframe that one has to think about is the 10-30 yr. The National Aeronautics and Space Administration (NASA), the national laboratories, the National Institutes of Health (NIH), and the National Science Foundation (NSF) are probably the only organizations in the country that deal with that. It is important not to apologize and reduce the reason for the existence of the national laboratories, NASA, and NSF to near-term technology transfer and things that we send to industry. It must be resisted. If we do that, we will be measuring our success by the yard—we do this little widget and that little widget—and be wasting the taxpayers' money. What we really do is rewrite chemistry, physics, and biology textbooks. We develop fundamental understanding of the laws of nature in the physical sciences and the biologic sciences so that corporations years from now can deal with them. We inspire young people to learn the scientific process and to become part of society.

The world economy today is $30 trillion a year, of which about 75% is represented by countries that can be counted on the fingers of 2 hands. The world economy is going to be $100 trillion some decades from now, and the growth is going to be in the developing countries. George David, CEO of United Technologies, in a recent speech to NASA calculated exports from the United States at $24 per hour; for imports, it is $3 per hour. If America thinks that it is going to put up big boundaries at its borders, continue to make sneakers and other low-end technology products, and assemble the products of yesteryear, the message is that it is hopeless at an average of $24 an hour. America has to drive up the food chain, be at the intellectual top, and add the value that will protect the economy, lead to sustainable development for the world, and provide national security, which is absolutely essential.

The General Electric corporation, a decade or two ago, got 75% of its jet-engine business from the federal government, and 25% from the commercial sector. Today, it is reversed. If America does not lead the world in the technology of materials, fluid flow, and computational fluid dynamics, not only will it have economic problems, but its national security will be at stake. General Electric lacks the resources to do that. Suppose that the chairman, Jack Welsh, went to the bank and said, "I would like for you to give me a loan because in 15 yr I would like to build a hypersonic engine. I do not know whether it will work, and the engine might be built of ceramic matrix instead of metal so that it will have much higher combustion efficiency, and maybe in 5 or 10 yr I will know whether I even have a chance of doing it. I need a few billion dollars." They would throw him out on his head. How else

does it get done but by the federal government? We do high-risk, high-potential-payoff research that will affect the future of this country in the long term.

When I go to Congress to testify, I refuse to explain the reason for NASA as Teflon, Velcro, and Tang. First of all, we did not invent any of it. And I cannot say, if you put a dollar into NASA today, a product will come out the back end. What I do say is that NASA tackles 7 basic questions. I think that this is a problem of communication for the Department of Energy (DOE) also, because no one knows what it is and what it will be in the country of the future. We have developed a strategic plan at NASA. It says exactly what we do. The new issue has 7 questions in it. Someone who does not work on these 7 questions should not be at NASA.

1. How did the galaxies, stars, solar system, and planetary bodies form and evolve, and how does this knowledge help us to rewrite chemistry, physics, and biology textbooks?
2. Is life of any form, single-cell or higher, carbon-based or not, peculiar to Earth?
3. How does knowledge of the planets and the stars help us to build predictive environmental, climate, and resource models to explain the interaction of the oceans, the atmosphere, the biomass, and the land and to separate out the effect of the human species?
4. How do we use the uniqueness of space—that is, the absence of gravity, the presence of partial gravity—to understand the laws of nature, and how do we open the space frontier by understanding how we could put people and robots into that environment?
5. How do we develop revolutionary technologies to make transportation in air and space—anywhere, any time, for anyone—safer, more economical, and more environment-friendly?
6. What tools do we need to answer questions 1-5, which are fundamental scientific questions, and how do we transfer these tools to enrich the American economy? (This question deals with technology transfer.)
7. How do we communicate this knowledge to the American people, especially to children to tell them that adults care about their future?

NASA is a science-driven agency that develops technology to support the science mission. Unless people can explain their relationship to those 7 questions and prove that they are the best in the world at it, we shut down their program. That might be shocking in light of the jobs that are put into specific congressional districts.

We are setting up centers of excellence because we had tremendous overlap. We found that at NASA we had 10 centers with marketing teams that were trying to justify their existence. Now, we are stopping that activity. We only justify what we do on the basis of answering the 7 questions. I went down to the NASA establishment in Mississippi, where they test rocket engines. I said, "Do you have the best rocket-engine testing facility in the world?" They said no. I said, "Where does it exist?" They said nowhere. I said, "How many rocket test facilities does America have?" They said 7. I said, "Why is that the case?" Because we cannot figure out the politics of shutting down 6 of the 7. Think about that and see whether it applies to what you do.

This is killing science in America. The Cold War is over. We are not going to spend whatever it takes to answer fundamental questions. Egos have to be set aside. We have to give young experimenters room to break through the system, not lock them out.

The peer-review system is wonderful, but the peer-review system maintains the status quo. For example, NASA was given the job of Mission to Planet Earth to answer question 3. We set up a team of people in the late 1980s and gave them multidecade grants. That is very bad. The team set up a system of building 3 generations of spacecraft that would be launched every 5 yr and that would cover a 15-yr period. The spacecraft had 24 sensors. They weighed 40,000 lb, were 15 ft in diameter, were 40 ft long, and cost billions of dollars. If a single sensor failed, they would have to be replaced. Before you launched the second, you would be building the third. We had a nice white-collar jobs program. You should have seen the intellectual outpouring when we announced that we were killing it. A total of $18 billion was allotted in this decade, and we now have it down to $6 billion. We had 2 spacecraft with 24 sensors launched: an a.m.-crossing spacecraft and a p.m.-crossing spacecraft. We would have launched these every 5 yr. Now we have about 16 or 18 spacecraft. What we say now is that you cannot build a spacecraft in more than 3 yr.

We do not even specify the science on the spacecraft when we go out for peer review. It is called the Earth-Space Science Systems Probe. Anyone in America can bid. They have to form a team and figure out the science

question that feeds into the broader question. They propose the science, the scientific team, and what they are going to build. The principal investigator dominates it. Every other year, we award 3 contracts. The total cannot be more than $240 million, and launch has to take place within 3 yr. We give 2 prime contracts, and we hold back a third. If one of the 2 teams does not do what it says it is going to do, we cancel the contract and give it to the third one. That is what I call peer review. It is a lot different from ordinary peer review. Young people are actually winning some of these things. We are trying to change NASA, but it is difficult. People speak with tremendous passion to hold onto the past, but the past is not there.

We were finding ourselves doing near-term work, and it raised the question of applied research versus basic research. I view it differently. It is near-term (0-5 yr), as industry handles product development, versus wide-open research, which can be in science or technology. We are forcing our budget more and more into high-risk, high payoff research.

Instead of building multibillion-dollar platforms, we are building much smaller sizes and in much higher quantity, so we are allowed to fail. In fact, we reward people when they fail. They cannot be incompetent or malicious, but we want people to take risks. We think that we can build some spacecraft in 1-5 yr from start to launch—and we are developing launch vehicles that cost $1.5 million, which is quite interesting.

I have been appalled by the scientific cannibalism in this country, a problem of the Cold War era: people somehow believe that if someone else's project is canceled, their own research will be covered. But the scientists of America are like sheep going to slaughter. Members of Congress love it when scientists attack each other's projects because once they are canceled, the money for them does not go into science, but goes into the survival portion of the American budget.

I am proud to say that I was a major supporter of the Superconducting Super-Collider, and my people said, "How can you support it? If you support that, we will lose the space station." I said, "How can I not support it? We have to study inner space while we study outer space because when we have telescopes powerful enough to look at some of the high-energy objects at the outer bounds of the universe, we are going to need Superconducting Super-Collider data on fundamental interactions of matter in inner space. It hurt when we lost the Superconducting Super-Collider.

Scientific cannibalism better stop, and we ought to go to real peer review, not nonsensical peer review of survival. It is killing American science, and it is killing our credibility; the scientific community is doing it to itself, and it grieves me.

After we developed the 7 questions at NASA, we developed 4 enterprises to address them: space science, earth science, humans in space, and aeronautics. Each enterprise gets questions to answer. For example, the space-science enterprise answers questions 1 and 2.

And I asked the program people to set up long-term goals so that the American public has a score sheet and knows what the enterprises are going to do. One of the goals is, within 10-15 yr, to detect Earth-size planets within 100 light-years of Earth, if they exist; and if they have an atmosphere, determine whether that atmosphere has oxygen and carbon dioxide in a chemical equilibrium with water vapor. If that is the case, we can infer photosynthesis. That is a goal; the American public has a score sheet and understands that. In the aeronautics enterprise, we adopted goals of reducing the crash rate of planes by a factor of 5 in 10 yr and by a factor of 10 in 20 yr, and so on. By next year, each of our enterprises is going to have a set of specific goals for 10 yr and 25 yr that are related to the 7 basic questions. We will reach some and we will fail to make some, but the goals will be clear. That will be our communication tool, so America will understand what we are doing and we will not apologize that we exist for Tang, Teflon, and Velcro. It is deadly and it is insulting to our scientists who try to justify their existence on the basis of near-term benefits to America.

We in government have not undergone the revolution that American industry has undergone through reinvention and continuous improvement. Industry's pace of doing things is lickety-split.

Ed McCracken, the CEO of Silicon Graphics, told me that its oldest product is 5 months old. Compare that with the pace of government in putting in a major engineering or scientific facility. We allow the bureaucracy to get in our way, and it is shameful. The problem is not in Congress; it is in the agencies and how they hide behind paperwork and have not dealt with the tough issues that have to be dealt with. We must get the bureaucracy out, and we must put the scientists and engineers back to work and not have them pushing paper and managing

contracts. When I came to NASA, NASA called them contract managers. These are people with doctorates from the best schools in the country—and even they could not get the development time down. In 1992, the average time for developing a spacecraft was 8 yr. Today, it is 4.5 yr. Our goal is an average of 3 yr by the end of the decade and about 2 yr, with a maximum of 3 yr, somewhere around 2005.

We are concerned with how much an average spacecraft costs to build and to launch, and we are not the only ones. It is time for some of the other science organizations to practice more openness because the American public is demanding it; it will allow us to have much better facilities. In the end, no corporation could afford to put in the kind of scientific and engineering facilities that the national laboratories have. They look on those facilities as a resource.

The Boeing Corporation and McDonnell-Douglas tell me that without NASA—our computational fluid dynamics and our wind tunnels—they could not do their job. Many American corporations rely on the national laboratories, including the DOE facilities, but our infrastructure is rotting. We are not maintaining the infrastructure, and we are accepting the nonsense that it is socially unacceptable to invest 20-30 yr out into the future. We are part of the problem because our development time for major new facilities is much too long, and we are not matching Silicon Graphics and turning them around in 5 months.

The public-private issue is also of concern. It is crucial that the government get out of the operations business and hand it over to industry. We intend to hand the keys of the shuttle over to a private corporation. We started the process. The amazing thing is that we are saving billions of dollars. Many people who have jobs on the shuttle are upset, so they hide behind the safety. You will see me getting beaten in Congress over this, but we took $1 billion a year out of the shuttle, and the reliability is 3 times what it was before, and 8,000 fewer people are working on it. It is not the goal of the federal government to support people in luxury through the year 2030 because they are working on the shuttle; it is the role of the federal government and NASA to answer the 7 questions that we developed.

I love people, and I do not want to come across as harsh, but I put my hand on the Bible and swore to do the right thing for NASA. The right thing is not to hug the shuttle. Everyone is saying, "How could you hand the shuttle over to a private company?" I say, "How would you like to fly on American Airlines managed by the federal government?" We laugh, but the very same people are trying to tell the American public that you have to protect jobs by having people work on the shuttle so that we will have people who watch people who watch people. And then, if the shuttle crashes, we will have all sorts of documentation so that we will say it is okay.

When I arrived at NASA, that shuttle would shut off at the Cape within milliseconds of launch. Here is how they fixed it: our prime contractor, Rocket-Dyne, added 8 more inspectors to look at something for which we did not have the fundamental process under control, to document it so that if we had a crash, they could say, by God, we are safe. In space, people could die. I repeat: in space, people could die, because it is tough and it is rough. You do everything you can do to make it safe, but it is not our job, for job security, to hide behind safety for the shuttle. We are going to hand the space station over to a private corporation and hand mission control over to private corporations because there is no need to have people with doctorates in physics, chemistry, and biology operating consoles where they look at analogue dials and turn knobs and are contract monitors. I think there are parallels in DOE.

I have dealt a little bit with ethics. I feel personally responsible for the lives of the astronauts. I feel that because of total quality management, continuous improvement, and re-engineering, the shuttle is a safer machine. Some 10 yr ago, America believed that the probability that the shuttle would blow up was 1 in 1 million. In 1992, the probability was 1 in 78. We have now worked our way back up to 1 in 248. I have an ethical responsibility to tell the American public that the space frontier is dangerous and that people might die. The head of public affairs at NASA dies whenever I say that emotionally. But there are other cases in which the ethical problem with respect to the American public is not that we do the wrong thing, but that we do not openly talk to the American public as though they are adults. We are so afraid of telling them the truth in the news media that we do stupid things. That is where we ran into the ethical problem of using people in nuclear experiments. In itself, it might not have been wrong, but that the American public was not told in advance was wrong.

How does that apply to NASA? We are going to send people out of Earth's orbit. When you leave Earth's orbit, you encounter cosmic radiation. The reason we have life on this planet is that these magnetic fields protect

us; the incoming cosmic particles are trapped in the Van Allen belts. When you go out there, you cannot put enough shielding in the spacecraft. The question is, How are we going to open the space frontier? We will have to pick some level of exposure above normal working levels. Could we go 3% more? 2% more? 1% more? We will have to talk openly about that.

We are going to have to deal with the ethics of the search for life. Right now, we are having open discussions with the religious community because we are using federal money and we do not want to offend people who have religious beliefs on this issue.

We are going to have to screen people on the basis of new knowledge about ourselves. We are still using very rudimentary techniques, but as genetic information increases, we will have to screen people genetically. There are going to be some interesting ethical questions. How do we deal with death in space? How do we deal with animals in space?

At NASA, we are trying to understand the relationships of the muscular, structural, and neurological systems. In a program called Bione, we were using primates. Some people feel animals should not be used in research. But I have a responsibility to make sure that astronauts will be healthy and safe when they go into space. A person who goes into space loses bone mass and muscle mass. If problems occur, we do not know whether bones will heal. So we used primates to try to find out, and we did not lose any primates until the last mission.

We had to do a biopsy on a primate when it came back. In the absence of gravity, everything in the body changes: the rate at which white and red blood cells are made changes, the immune system is depressed, fluids shift, bones decompose, muscles vaporize away, the vestibular system is lost, and so on. Unfortunately, with Bione, the scientists did biopsies on the primates 7 days after the mission. That is just the way they were doing it, as Tevye says, by tradition. We had to take the biopsies 1 day after they came back. But the primate did not adapt from zero gravity to 1 g, and it died. We had an ethical dilemma: we said we needed the data, but we would not take the data if doing so would put the primate at risk. It was a catch 22. So we said that we would stop the primate research at once because trying to obtain the needed data would cause us to kill the monkey, and then we would not be able to get the data. We try to deal with the ethics as well as we can. Now we are going to look for other methods of getting the needed information. We might find nonintrusive methods, and we might not.

What happens if an astronaut has appendicitis in space? What if you bring him or her back from space very quickly, roll up the ambulance, and take the astronaut to the hospital? Could you give an anesthetic? How do you handle medicine in space under zero gravity? We have a whole new set of issues. How do we determine what to do? We cannot just go at it scientifically. We have to look at the ethical boundaries. We have an ethics board to help us to make these decisions, but the key is that we are open with the American public.

NASA is probably one of the most open agencies. In effect, we have 2 billion people in our laboratories day and night. That is a strength.

I do not know as much about DOE as I should, but I have tried to share my own experiences. I encourage you to go back to your laboratories and start a revolution—try to repeat the lessons that American industry learned in the last 10 yr to change your processes, but never give up on long-term basic research, because the national laboratories are a jewel of this country. If we lose the national laboratories, I will worry about the future of our country. But we could lose the national laboratories unless they get focused and have a specific set of goals and questions that they can communicate to the American public so that the public can understand what they are doing.

I would like to leave you with one more thought. I have an engineering and physical-science background, and I am now seeing the error of my ways. When I went to school, biology was an art, not a science, in my mind. But the life sciences are becoming much more analytic. I see a major problem at NASA. Just after we discovered what we think are signs of fossilized life on Mars, we had a meeting of the Mars Working Group, 50 of the smartest scientists from academic institutions around the country. I asked, "How many life scientists are in the room?" Two hands went up. Mind you, we are searching for life.

We just held our 5-yr review in Boulder, Colorado, with 75 people who are looking at the origins, evolution, and destiny of life. We have improved: we had 6 biologists.

One of the problems in American science is that we train people through smokestacks. I do not propose that people lose their sharp skills by acquiring multidisciplinary skills, but they have to be able to talk across disciplinary lines. To prove my credibility, I went to the American Physical Society and lectured them that they are not

training physicists with a fundamental background in biology, although physics and biology are going to come together. Biologists do a better job with the physical sciences, but still not good enough. The next 10-15 yr will see an integration of physics, chemistry, and biology.

For NASA, it is essential. To that end, we are opening the Astrobiology Institute, in Sunnyvale, California. We want to understand the origin of life. We want to learn whether life is ubiquitous in the universe. This is getting down to the fundamentals of science, but I do not see young people being trained to have the capacity to design the instruments that we are going to send into orbit and to other celestial bodies.

In engineering, we are concerned about how little work is being done in self-assembling biologic systems, where we either mimic biology or use biology to build both living and inorganic systems. This is really the future of engineering. Engineers are ignorant on this subject. Biologists have an opportunity to be on the cutting edge and are not doing enough, are not communicating with physical scientists and engineers to bring this marriage about.

At NASA, we are going to try our best. I have to appoint a chief scientist, and I have waited a year. But I will not appoint a chief scientist who is not a biologist or other kind of life scientist, because we must restructure the agency.

We must be more forceful in helping people to understand that a crisis looms in our country. Very few people understand—although they hear about the Genome Project and drugs—that biology is going to affect everything we do. Almost no thought is going into bringing together the biologists, the engineers, the physicists, and the chemists.

Part V

Biological and Environmental Research Program Recognition Awards

Biological and Environmental Research Program Recognition Awards

On the last day of the symposium, 13 scientists who have made significant contributions to the advancement and progress of the BER program were honored. In presenting the awards, Dr. Patrinos read the inscriptions.

Dr. Mina Bissell, Lawrence Berkeley National Laboratory
In recognition of your sustained outstanding research conducted in support of the BER program in the area of molecular and cellular biology to understand how cell growth, differentiation, and survival are controlled in normal and cancerous breast cells.

Dr. Charles DeLisi, Boston University
In recognition of the seminal role you played while Associate Director of Health and Environmental Research in proposing and initiating the Department's, the nation's, and the world's Human Genome Program in 1986.

Dr. Jay Edmonds, Pacific Northwest National Laboratory
In recognition of your sustained outstanding research conducted in support of the BER program for global climate change to understand the environmental and economic consequences of carbon dioxide emissions, and for developing innovative models to assess the energy impact on climate.

Dr. Joanna Fowler, Brookhaven National Laboratory
In recognition of your sustained outstanding research conducted in support of the BER program for medical applications to create new concepts in medical imaging, and to design, synthesize, and apply radio-tracers to the study of the human brain in health and disease.

Dr. Lawrence Gates, Lawrence Livermore National Laboratory
In recognition of your sustained outstanding research conducted in support of the department's BER program for global climate change through the development of the methodology to inter-compare climate models to systematically ascertain and correct model biases and errors.

Dr. Joe Gray, University of California, San Francisco
In recognition of your sustained outstanding research conducted in support of the BER program in the area of health effects to develop molecular/cytogenetic tools, such as chromosome paints so valuable for clinical and research applications.

Dr. Michael Huston, Oak Ridge National Laboratory

In recognition of your sustained, outstanding research conducted in support fo the BER program for ecosystem functioning and response, by developing innovative concepts of the general patterns of biodiversity and how environmental change and human influences affect biodiversity.

Dr. Michael Knotek, Argonne National Laboratory

In recognition of your outstanding leadership in bringing to fruition the William R. Wiley Environmental Molecular Sciences Laboratory and National Collaborative User Facility for providing innovative approaches to meet the needs of the department's environmental missions.

Ms. Betty Mansfield, Oak Ridge National Laboratory

To recognize you as founding and managing editor for the Human Genome News, and for outstanding success in communicating scientific information to the U.S. and international community about the Department's BER program.

Dr. J. Craig Venter, The Institute for Genomic Research

In recognition of your sustained outstanding research conducted in support of the Department's BER program for determining the first three complete microbial genome sequences, discovering and cataloging new human and microbial genese, and exemplifying the private sector's collaborative role in federal programs.

Dr. Tuan Vo-Dinh, Oak Ridge National Laboratory

In recognition of your sustained outstanding research conducted in support of the Department's BER Program for instrumentation and measurement science to discover new concepts in analytical chemistry and to invent and transfer to the private sector technologies applicable to medical and environmental monitoring.

Dr. Warren Washington, National Center for Atmospheric Research

In recognition of your sustained outstanding research conducted in support of the BER program for climate research through the development and application of advanced coupled atmospheric, ocean, general circulation models to study the impacts of anthropogenic activities on future climate.

Dr. Edward Westbrook, Argonne National Laboratory

In recognition of your sustained outstanding research conducted in support of the Department's BER program for structural biology, to develop advanced detectors for crystallography, while providing leadership to established user facilities for structural molecular biology at the advanced photon source.